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RESEARCH HIGHLIGHTS

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RESEARCH HIGHLIGHTS

NATIONAL INSTITUTES OF HEALTH

1960

Items of Interest on

Program Developments and Research Studies

Conducted and Supported by the Institutes

and Divisions of NIH

As Presented to the

Congress of the United States

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service

INTRODUCTION

For several years the National Institutes of Health, main research center of the Public Health Service, has submitted statements supplementing its testimony at congressional hearings on appropriations to the U. S. Department of Health, Education, and Welfare. The following pages comprise the statements called Research Highlights as prepared by the Institutes and Divisions of NIH for hearings on the fiscal 1962 budget.

The compilation is intended to meet requests and administrative needs. It contains representative items selected in light of both scientific importance and public interest. In no sense is it a comprehensive review of NIH activities, nor even necessarily of its most significant results.

The items concern the work of both staff scientists (laboratory and clinical) and research grantees. No attempt has been made to reflect the relative magnitude of the grants program. During the period covered--calendar year 1960--more than three-quarters of NIH funds for research were awarded to investigators in universities, medical schools, and other non-Federal research centers.

The items in this report are based on published work. The scientists' articles appear in a wide range of journals, mainly non-Federal, which are available in medical libraries. Further information, including references to these articles, may be obtained through the Office of Research Information, NIH, or the information office of the Institute concerned.

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Public Health Service
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

HIGHLIGHTS OF PROGRESS

IN RESEARCH ON

CANCER

1960

Items of Interest on Program Developments and Research Studies

Conducted and Supported by the National Cancer Institute

The National Cancer Institute gave increasing emphasis to development and expansion of programs especially oriented to research on human cancer. At the same time, laboratory, clinical, and field studies contributed to the accumulation of knowledge necessary for a more complete understanding of malignant disease.

The Institute invested over $5 million in virus-cancer research grants. One such grant-supported study reported the discovery of a filterable agent that induced immunity in rats to six different tumors. This discovery re-emphasized the complex interrelationships that may exist between infectious disease and resistance or susceptibility to tumor development. Environmental studies in several states confirmed earlier reports of the higher incidence of cancer among urban compared with rural residents. Information such as this may lead to better understanding of the causes and, subsequently, more effective ways for preventing some kinds of cancer.

The Cancer Chemotherapy National Service Center is now sponsoring clinical trials of more than 150 drugs. One investigation reported successful use of a new hormone-like compound, delta-one-testololactone, in temporarily controlling, with no masculinizing side effects, the lesions resulting from the spread of breast cancer. A comprehensive review of meningeal leukemia suggested a treatment method. Other data on advanced refractory acute leukemia provided detailed new information on the natural history of this form of cancer.

The items presented in this report are representative of the highlights of program developments and progress in research conducted and supported by the National Cancer Institute. Institute staff scientists are identified by the Laboratory or Branch with which they are connected and grantees by their institution. Most of the results were reported by groups of investigators, but for the sake of brevity, only the senior author is identified in most of the summaries.

VIRUS RESEARCH

FURTHER STUDIES DESCRIBE TUMORS IN MICE RECEIVING POLYOMA VIRUS INOCULATION

The SE polyoma virus has become an important laboratory tool in the intensified research now under way to learn whether viruses cause human cancer. Dr. Sarah E. Stewart, Laboratory of Biology, and Dr. Bernice E. Eddy, Laboratory of Viral Products, Division of Biologics Standards, have reported extensively on their collaborative studies of the virus, which Dr. Stewart obtained from the tissues of leukemic mice.

Further studies on the development of tumors in mice inoculated with polyoma virus have been reported by Dr. Mearl F. Stanton, Laboratory of Pathology, and Drs. Stewart and Eddy. Swiss mice inoculated late in fetal life with tissue culture preparations of the virus developed a greater incidence of tumors and died earlier than mice injected shortly after birth. However, no type of tumor was found that had not been previously observed in mice inoculated as newborns.

Mice inoculated as newborns from mothers exposed to an environment contaminated by the virus had a significantly longer life-span and lower incidence of salivary-gland tumors than mice inoculated as fetuses in contaminated mothers or as newborns from uncontaminated mothers.

MOUSE MATERNAL ANTIBODIES IN MILK AND SERUM INDUCE POLYOMA VIRUS RESISTANCE

Drs. Stewart and Eddy reported that the resistance which mice develop to tumor induction by the SE polyoma virus was traced to the presence of maternal antibodies in milk and serum.

Milk taken from nursing mothers that had been exposed to the virus and from those not exposed showed pronounced differences in antibody titers. Mice of the maternal strain that were housed in the same room with mice that had received polyoma virus had serum antibodies specific for the virus. Pooled sera from 12 breeders completely inhibited tumor induction in 24 mice given sufficient virus to cause tumors in all of 16 mice that did not receive serum.

The range of antibody titers of pooled milk from exposed mice, as shown by tests on the inhibition of hemagglutination of guinea pig red blood cells, was similar to that observed in the sera of exposed mice.

NO CORRELATION OBSERVED BETWEEN POLYOMA ANTIBODY AND LEUKEMIA IN MICE

Dr. Lloyd W. Law, Laboratory of Biology, has collaborated with scientist's of the National Institute of Allergy and Infectious Diseases, in a study of the relation of polyoma virus infection to lymphocytic neoplasms in mice.

Leukemic and non-leukemic mice of four high leukemic strains were tested at intervals for antibody to polyoma virus. No correlation was found between the presence of detectable antibody and the appearance of leukemia at various times throughout life. No evidence was observed that mice with circulating antibody against polyoma had an increased risk of developing leukemia. The relationship between antibody status and recovery of virus from organs of leukemic mice was found to be similar to that previously observed in non-leukemic mice of infected colonies by Dr. Wallace P. Rowe, National Institute of Allergy and Infectious Diseases, and others.

The results suggest that any association of polyoma virus and experimental transmission of leukemia is fortuitous.

STUDY CONFIRMS LODGING OF POLYOMA VIRUS CHIEFLY IN INFECTED CELL NUCLEI

Dr. Richard A. Malmgren, Pathologic Anatomy Branch, has reported results of a study strongly suggesting that the polyoma virus localizes primarily in the nucleus of infected cells.

Polyoma virus was grown in cultures of milk-adapted mouse lymphoma cells. The infected cells were exposed to labeled antipolyoma virus serums. These had been obtained from rabbits immunized against the virus and had been labeled with fluorescein isothiocyanate. The cells were fixed on slides and examined under ultraviolet light. Clumps of fluorescent material of varying size were scattered throughout the nucleus and were particularly prominent near the inner surface of the nuclear membrane. These fluorescent particles were not seen in uninfected tissue-culture preparations.

Since the deposition of dye-labeled antivirus serums is dependent on an antigen-antibody combination, the finding is interpreted as demonstrating either virus or virus-associated antigen in the nucleus of cells infected with polyoma virus.

Cytochemical studies reported by other National Cancer Institute investigators have demonstrated abnormal nucleoprotein in vacuoles within the nucleus of polyoma virus-infected cells. The present finding is consistent with these observations.

Electron microscope studies at the National Cancer Institute have also demonstrated large collections of viral particles within the nucleus of infected mouse lymphoma cells. However, the electron microscope studies also showed viral particles in the cytoplasm of infected cells. It is suggested that the concentration of viral particles in the cytoplasm may be too small to be revealed by the fluorescent antibody technique.

ROUS VIRUS PINPOINTED IN SARCOMA CELL CYTOPLASM

Dr. Malmgren and his associates in other laboratories of the National Cancer Institute have also reported results of a study designed to pinpoint the location of the Rous sarcoma virus within the cells of the Rous sarcoma. Electron microscope studies previously reported in the scientific literature demonstrated particles thought to be the Rous sarcoma virus in the cytoplasm of sarcoma cells.

Chicken and turkey anti-Rous sarcoma virus serums obtained from fowl in which Rous sarcomas had regressed were labeled with fluorescein isothiocyanate. When sections of sarcoma tissue were exposed to the fluorescein-labeled anti-Rous virus serums, fluorescence was observed in the majority of instances in the cytoplasm of sarcoma cells. Occasionally, intranuclear fluorescence was noted. The number of sarcoma cells containing the fluorescent material was related to the amount of virus inoculated into chickens to initiate tumors.

Fluorescence was not seen in the cells of normal chicken tissues exposed to the labeled anti-Rous sarcoma virus serums.

TISSUE CULTURE TECHNIQUE FOR ASSAY OF TUMOR VIRUS DEVELOPED IN GRANT STUDY

A possible new tool for approaching the problem of studying viruses as causative agents in cancer is suggested by recent findings in in vitro studies of the chick embryo--Rous sarcoma virus (RSV) system. Dr. Harry Rubin, University of California, Berkeley, reported that the studies pointed the way to the use of RSV for assaying lymphomatosis virus, which does not produce overt cytopathic effect in vitro, in cultures of chick embryo cells.

The study had its origin in the observation that resistance to infection with RSV occurred sometimes in chick embryo cultures. The resistance was found to be associated with the presence of one or more embryos whose cells were found to be resistant when cultivated separately.

An agent, named resistance-inducing factor (RIF), was isolated

from the medium of resistant cultures. RIF multiplied extensively in infected cultures and could be measured quantitatively. It was sedimented in the ultracentrifuge under conditions adequate to sediment RSV, and could be neutralized by antiserum to RSV (obtained from chickens that had recovered from tumors induced with RSV).

These characteristics, as well as the widespread occurrence of RIF in eggs, suggested that RIF is a virus of the avian lymphomatosis complex. Support for the latter relationship was found in the observation that four strains of lymphomatosis virus mimicked the action of RIF by inducing resistance to RSV in chick embryo cultures.

This finding led Dr. Rubin to suggest that the technique--in which one virus acts as a specific inhibitor of the growth of another--might be adapted to the detection of viruses associated with other malignancies.

A NEW FILTERABLE AGENT INDUCES IN RATS IMMUNITY TO SIX DIFFERENT TUMORS

A new method of inducing immunity against several widely employed transplantable rat tumors has been reported by Dr. Jerome H. Sacks and his colleagues at the Medical College of Virginia. The immunizing material, which was discovered in the investigators' laboratories, is an infectious replicating agent and produces in rats an acute hemolytic anemia.

At the time the report was prepared, this agent (FHA, filterable hemolytic anemia) had been transmitted serially in over 140 passages in some 2,500 rats and had remained stable both in its infectivity and the hemolytic syndrome it produced. The hemolytic disease is transmitted by inoculation of any of a variety of tissues or fluids taken from an animal at the height of the disease. For example, 1/100,000 ml of serum administered intraperitoneally is sufficient to infect normal animals. FHA is strongly antigenic and stimulates the production of neutralizing antibodies. It is extremely small--filterable through filters with pore diameters of 20 mμ--and is nonsedimentable in the ultracentrifuge even under such drastic conditions as 144,000 g (units of gravitational pull) for 10 or 12 hours.

In the present study, 60 (54 percent) of 111 FHA-immune rats were resistant to transplantation of six different tumors; 7 (6 percent) of 114 control rats receiving transplants of the same tumors remained tumor-free. FHA-immune rats were those that had recovered from the hemolytic disease following intraperitoneal inoculation of infectious whole blood.

Some FHA-immune rats that developed tumors survived three times as long as the controls. In others, the tumor that developed was altered in growth pattern, gross appearance, and histologic morphology. The changes, never observed in control animals, included encapsulation without infiltration, extensive necrosis, and a more differentiated appearance. These results are interpreted as manifestations of heightened host resistance.

Additional evidence demonstrating the presence of humoral antibodies in animals recovered from infection with FHA was found in experiments in which tumor cells were incubated in the test tube with sera from FHA-immune animals. The sera were extremely toxic for the tumor cells, but not for normal cells present in the mixture. Control sera did not produce cytotoxic changes in either normal or tumor cells.

Dr. Sacks believes the findings suggest that the tumors studied share a common antigen with the FHA agent not possessed by normal rat tissues.

MOLONEY MOUSE LEUKEMIA VIRUS STUDIES SUMMARIZED

Dr. John B. Moloney of the Laboratory of Biology, has reported a detailed study of a virus that induces lymphocytic leukemia in mice. His first report of the isolation of this virus from transplantable sarcoma 37 was made a year ago.

This virus is characterized by the following biological and physical properties: 1) infection by the agent is not limited to the very young or to mice of a single genetic type; 2) the virus-induced lymphocytic leukemia is similar to the spontaneous type of the disease; no other neoplasms have been observed in the test mice regardless of strain used or age of the recipient at the time of virus inoculation; 3) the high tumor-producing activity of the virus concentrates induces leukemia in essentially 100 percent of the inoculated mice within a relatively short latent period--2.5 to 3 months; 4) as observed in the electron microscope, by Dr. A. J. Dalton, also of the Laboratory of Biology, the virus particles measure 65 to 100 millimicrons in diameter; and 5) the agent is stable to freezing and lyophilization.

The high biological activity, stability, and absence of strain specificity indicate that this virus may be a valuable addition to the group of tumor viruses now available for laboratory investigations.

TRANSPLANTS OF VIRAL LEUKEMIA TISSUE INDUCE SOLID TUMORS IN MICE

A "leukemia-like disease" in mice, induced by a virus and transmissible by cell-free filtrate, was described several years ago by Dr. Charlotte Friend at the Sloan-Kettering Institute for Cancer Research. The view of the neoplastic character of this disease was challenged on the grounds of inability to produce tumors by transplantation of leukemic cells. Drs. Rita F. Buffett and Jacob Furth, at the Children's Cancer Research Foundation, have now reported success in producing transplantable reticulum-cell sarcomas, originating from the systemic disease induced by the Friend virus.

The tumors arose in mice receiving subcutaneous or intramuscular grafts of liver fragments from animals infected with the virus. Tumor transplantation was facilitated by pre-irradiation of test animals.

The tumors grew locally, attained large sizes, and could cause death, with or without generalized leukemia. They closely resembled autonomous, supposedly virus-free reticulum-cell sarcomas. A number of the animals that developed tumors also had enlarged spleens characteristic of the generalized Friend leukemia, suggesting the presence of the virus in some of the tumor-bearing hosts.

PRECANCEROUS MAMMARY TISSUES OF MICE SHOW VIRUSLIKE PARTICLES

The results of many electron microscope studies reported in the scientific literature have shown viruslike particles in tissues of mice known to carry the mammary-tumor virus. Yet, unequivocal proof of the identification of the particles with the biologically active virus is lacking. Dr. Dorothy R. Pitelka and her associates at the University of California, Berkeley, have now reported new evidence to support the theory that such particles are identical with the mammary-tumor virus.

Earlier studies in this laboratory had shown that viruslike particles were abundant in tumor tissue and hyperplastic alveolar nodules of C3H/Crgl mice, a strain that has a high incidence of mammary tumors, and that the hyperplasias were preneoplastic.

The present study has confirmed the consistent occurrence of these particles in hyperplastic mammary tissues of this mouse strain; it has shown the occurrence of these particles in hyperactive outgrowths of nodules transplanted into the gland-free mammary fat pads and in similar hyperplastic tissues from

four C3H sublines that presumably lack the mammary-tumor virus.

On the other hand, hyperplasias that are not preneoplastic, such as prelactating lobules of pregnancy, outgrowths from normal transplants, and "noduloids" induced by the administration of hormones, contained only rare, scattered particles.

The investigators conclude from these findings that "a clear and consistent association of viruslike particles with precancerous mammary hyperplasias in C3H mice is established."

INSTITUTE INCREASES SUPPORT OF RESEARCH ON VIRUSES AND CANCER

National Cancer Institute grants for research on the possible relationship of viruses to cancer, including cancer in man and experimental animals, totaled over five million dollars during 1960. Many of the grantees are virologists who have recently turned to long-term cancer research.

At several meetings, members of the Viruses and Cancer Panel of the National Advisory Cancer Council stressed the growing demand for research materials needed in the tremendously increased program of virus-cancer research. How to meet the first need--standardized cultures of living cells for the study of viruses suspected as cancer agents--has now been worked out. A national cell culture collection coordinating committee was established to develop criteria for certifying and preserving strains of mammalian cells of interest in cancer research. Well over a dozen laboratories in the United States are at present interested in assisting with this effort and the American Type Culture Collection in Washington, D.C. is to serve as a repository and distribution center for such standard cell strains as are needed by investigators.

In order to meet another need of expanded virus-cancer research, the National Cancer Institute awarded a contract to the University of Kansas Medical Center to develop detection agents which will identify some of the many viruses found in the human digestive tract. The work of the Kansas Medical Center laboratories had previously been developed by National Foundation support in their polio studies. Other contracts for detection agents to identify other groups of viruses are expected to be awarded in the future. This is a vital first step necessary before determination of any causal relationship between these viruses and cancer in man.

Additional needs of virus-cancer investigators which members of the Viruses and Cancer Panel hope will be met with the assistance of the National Cancer Institute include: 1) development of a laboratory animal (probably the mouse) whose viruses and genetic characteristics are known; 2) detection agents for identifying viruses present in such animals; 3) increased availability of monkeys and other primates for studies of human viruses suspected of causing cancer; 4) provision and distribution of an adequate supply of human normal and malignant tissue and blood; and 5) design and production of special research equipment, such as a liquid nitrogen refrigerator for indefinite preservation of living cells at extremely low temperatures.

Summer institutes for scientists in fields related to virus research and more extensive use of present training programs have been suggested as ways of stimulating interest in the virus-cancer field and enlarging the number of trained investigators. Distribution of translations of foreign scientific papers and more special symposiums on current problems will help to meet a recognized need for improvement in communication in the fast-moving area of cancer virology.

ENVIRONMENTAL CANCER

NEW YORK STATE CANCER INCIDENCE PATTERN SAME AS FOR IOWA, CONNECTICUT

Scientists of the New York State Department of Health and the National Cancer Institute have collaborated in an epidemiological survey of cancer incidence in urban and rural areas of New York. The data, reported by Dr. Morton L. Levin, now at the Roswell Park Memorial Institute, and William Haenszel, Biometry Branch, National Cancer Institute, were obtained for the period 1949-1951 from a central cancer register maintained by the Department of Health. All physicians, hospitals, and laboratories in the State (exclusive of New York City) report cancer patients to this register.

The cancer risk was generally greater for the urban than for the rural population. Urban-rural ratios of incidence rates were higher for males than females. The largest urban-rural ratios noted for males were for the respiratory system, buccal cavity and pharynx, esophagus, intestine, rectum, and urinary organs; for females, buccal cavity and pharynx, and urinary organs. The findings showed substantial agreement with the incidence rates and urban-rural ratios of rates reported from Connecticut and Iowa.

CANCER MORTALITY RATE AMONG NUNS AND WOMEN IN GENERAL COMPARED

Previous studies have suggested that women in religious orders have a higher risk of developing cancer of the breast and a lower risk for cancer of the uterine cervix than other women. Dr. Ruth S. Taylor and her associates in the Field Investigations and Demonstrations Branch, have now reported results of a study comparing mortality among three orders of Roman Catholic nuns with that of a group of women in the general population of similar age and background, with particular reference to cancer. The study was designed to detect any differences that might be attributed to the single as contrasted to the married state, and to obtain definitive information concerning total cancer and some of the major causes of death other than cancer.

The findings of the present study with respect to breast cancer are in general agreement with those of previous studies, all of which indicate that single women have a lower risk of mortality from breast cancer early in life, then increasingly higher rates, with a lifetime rate that is much higher than that of married women.

The results also confirmed a mortality risk for cancer of the uterus that is much higher among married than among single women. The evidence indicates that the difference is due to a deficiency in the rate of cancer of the uterine cervix among single women.

The Sisters showed lower total cancer mortality than did the controls between the ages of 20 and 59, higher rates above that age, and total experience about the same. They showed a much higher mortality rate from tuberculosis than did the controls, the highest risk occurring between the ages of 20 and 29. In mortality from cardiovascular-renal disease, the Sisters had more favorable experience than did the controls, particularly after age 50.

CIGARETTE SMOKE MAY PLAY PHYSICAL, NOT CHEMICAL ROLE IN CANCER OF LUNG

Dr. Paul Kotin at the University of Southern California School of Medicine, Los Angeles, has published a report on the role of cigarette smoking in the development of lung cancer.

Laboratory analysis was made of the content of aromatic polycyclic hydrocarbons in the mainstream smoke, which consists of aerosols respired by the smoker, and in sidestream smoke, which represents the smoke from the burning end of the cigarette.

The 3,4-benzpyrene content was of particular interest because it is a well-known carcinogen (cancer-causing substance) for animals and is present in considerable quantity in cigarette smoke. The carcinogen was detected in the condensate collected in 8 puffs as follows: mainstream smoke, 35 to 50 micrograms per cigarette; sidestream smoke, 135 micrograms per cigarette. There was, thus, greater production of the carcinogen during the period between puffs than during the time of actual smoke inhalation.

Other laboratory studies were conducted to determine the effect of cigarette smoke on the physiological activity of the respiratory epithelium. Isolated epithelial segments were exposed to aerosols containing cigarette smoke products in a specially designed chamber. Three phases of response were observed in the flow of mucus on the epithelium: first a sharp increase, which reached a peak in a few seconds, then a sharp decrease of about 16 minutes' duration, and finally a recovery period of about 30 minutes' duration. When epithelium was exposed repetitively to simulate smoking conditions, the inhibition or slowing of mucous flow was intensified.

It was concluded that the data confirm results of numerous other laboratory and epidemiological studies showing that cigarette smoking is a causative factor in the increasing incidence of lung cancer. However, Dr. Kotin disagrees with the conclusion that it is the presence of certain carcinogenic hydrocarbons in the smoke which explains its role in the development of lung cancer. Rather, he suggests that cigarette smoke is a potent irritant of the tracheobronchial epithelium. The irritant effect is wholly unspecific and is capable of being produced by numerous other environmental irritants that differ from cigarette smoke both in chemical composition and physical properties. The adverse effect of cigarette smoke is produced by impairing ciliary action, slowing mucous flow, facilitating prolonged retention of particles, and increasing cellular permeability.

Dr. Kotin's work was supported in part by grants from the National Cancer Institute and the Division of General Medical Sciences. The paper is the second in a series on the relative etiological significance of several environmental factors that may be associated with the development of lung cancer. In the previous paper, the role and mechanism of action of air pollutants were discussed.

FURTHER STUDIES SHOW CHROMIUM COMPOUNDS ARE CARCINOGENIC FOR MICE

Dr. William W. Payne, of the Environmental Cancer Section, has reported further evidence of the carcinogenicity of chromium in laboratory animals. The results were obtained in a continuing study to learn whether chromium in one or more forms is responsible for the increased rate of lung cancer among chromate workers.

About a year ago, in collaboration with Dr. Wilhelm C. Hueper, Head of the Environmental Cancer Section, Dr. Payne reported the development of cancers in rats that received implants of moderately soluble chromium compounds. The present study was undertaken to determine whether the compounds would produce similar results in another species, the mouse.

Mice receiving intramuscular implants of calcium chromate and sintered calcium chromate (prepared by heating pure calcium chromate to approximately 2000° F. for about an hour) developed tumors at the site of implantation. Sintered calcium chromate produced more tumors than did calcium chromate. The tumors, which began to appear seven months after administration of the carcinogen, were sarcomas of the spindle cell or fibrosarcomatous types.

DOGS FED LARGE QUANTITIES OF AN INSECTICIDE DEVELOP CANCER OF THE GALLBLADDER

The development of gallbladder and bile duct adenocarcinomas has been reported in dogs fed massive, toxic quantities of an insecticide, Aramite, over a long period. During a period of 46 months, 28 dogs received from 500 to 1,429 parts per million of Aramite per day. Fifteen of 19 animals autopsied after 462 to 1,220 days had one or more well differentiated adenocarcinomas in the bile ducts and gallbladder. This effect was different from that in rats and mice, which develop liver damage and in some instances liver cancer.

Aramite, a mixture of two closely related organic sulfites, is a commercial insecticide, especially effective as a miticide, and is manufactured by the Naugatuck Chemical Company, a division of U. S. Rubber Company, Naugatuck, Connecticut. This material was undergoing a series of tests by the investigators who made the present report to determine whether residues of one part per million on certain food crops were safe for human consumption. The Federal Register of February 12, 1960 (Volume 2, page 17, Pesticide Regulations) lists the establishment of a zero tolerance for Aramite for a long list of fruit and vegetable crops.

The study was made collaboratively at the Memorial Center for Cancer and Allied Diseases, Mount Sinai Hospital, New York City; and Food and Drug Research Laboratories, Maspeth, New York. It was reported by Dr. Stephen S. Sternberg and his associates.

DIAGNOSTIC RESEARCH

DIAGNOSTIC RESEARCH SEEKS PROCEDURES FOR DETECTING UNSUSPECTED, EARLY CANCER

Since early detection of cancer is of vital importance, the National Cancer Institute has in progress a program of intensive research in diagnosis, with the objective of devising procedures that will reveal the presence of unsuspected, early cancers. Established two years ago, the program employs a broad interdisciplinary approach and is being carried out through contractual arrangements, direct cooperative activities, and research grants.

As of October 15, 21 contracts in the amount of $1,019,000 and 68 research grants totaling $1,101,000 had been awarded to investigators working toward the development of a number of methods for detecting cancer in its early stages. These studies are based on the recognition of cancer as a group of diseases rather than a single disease and therefore look forward to the development of several diagnostic procedures.

Some of the new studies are concerned with correlating the presence of certain enzymes in the blood or urine of a patient with the existence of specific cancers. Other investigations are designed to determine the relationship between cancer and protein patterns observed in body fluids and secretions. Variations in hormone patterns between cancerous and noncancerous individuals are also under study.

Preliminary tabulation of the Institute's series of studies of vaginal specimens obtained from over 600,000 women and examined by the cytologic technique confirms earlier reports of the value of this method as a means of detecting early cancer of the uterine cervix in large population groups. Evaluation of the data should also produce information on the natural history of this form of cancer.

More than 3 million women each year receive cervical cancer examinations by the cytologic technique. A large percentage of them are obtained from personal physicians. It is believed

that the systematic application of the cytological examination to the total female population followed by treatment as indicated could result in the virtual elimination of cancer of the cervix as a cause of death.

With this encouragement, investigators are intensifying their search for ways to adapt the cytologic method for the study of cancer of other body sites. Five of the 11 direct cooperative research studies in the current diagnostic program are concerned with the use of cytology as a method for detecting cancer of other sites including the digestive tract and genito-urinary system. Cost of all 11 direct cooperative research studies is estimated at $663,000.

CHEMOTHERAPY RESEARCH

CLINICAL TRIALS SHOW ALKALOID, VLB, PROMISING AGAINST CHORIOCARCINOMA

In previous studies in the Endocrinology Branch, Vincaleukoblastine (VLB) was found to inhibit human choriocarcinoma, a rare, highly malignant tumor of the uterus arising during or after pregnancy, transplanted into the cheek pouch of hamsters. On the basis of this observation, eight women with the disease were treated with VLB by scientists of the Endocrinology Branch. All of the patients had developed resistance to methotrexate and to several other antitumor agents.

Vincaleukoblastine is an alkaloid originally derived from the periwinkle plant in 1957 by Cutts, Beer, and Noble, of the Collip Research Laboratories, University of Western Ontario, London, Ontario, Canada. The anti-cancer activity of VLB in mice was also investigated by Dr. Irving S. Johnson of the Eli Lilly Company. The first clinical trials were carried out simultaneously at Indiana University Medical Center, at Ontario Cancer Institute, and at the National Cancer Institute. The drug was provided for research purposes by Eli Lilly Company.

In the present study, reported by Dr. Roy Hertz, the eight women received intravenously one to six three-day courses of VLB in varying amounts of 9 to 36 mg. per course. Therapeutic effect was determined by reduction in the size of metastatic lesions and in the level of urinary gonadotropin, a hormone produced by the tumor.

Of the eight women treated, one showed complete remission, which at the time the report was prepared had lasted six months. In two others the urinary gonadotropin excretion reached normal levels. Two patients showed regression of pulmonary metastases, and three had no response following administration of VLB.

Some of the side-effects observed in all patients were suppression of the bone marrow when total VLB dosage reached 30 mg., hair loss, and severe mental depression. Inhibition of the deep tendon reflexes occurred in five patients and fever in six. All toxic effects proved reversible following each course of therapy.

Results of other clinical trials reported in the scientific literature suggest that the potentialities of VLB should be explored further for the treatment of acute leukemia and Hodgkin's disease.

SARCOLYSIN PROMISING AGAINST VARYING TYPES OF MALIGNANT TUMORS

Dr. Bernard J. Miller of Jefferson Medical College has reported results of a study showing that favorable effects can be obtained in patients by administration of a derivative of nitrogen mustard, commonly known as sarcolysin.

A group of 60 patients with far-advanced malignant tumors were given the drug orally (53 patients) or by the isolation-perfusion technique, usually in a dosage of 2 milligrams per kilogram of body weight. Objective evidence of favorable response was a gradual regression of tumor masses, occurring within two to four weeks.

Sarcomas of varying types, especially Hodgkin's disease, were most susceptible to the drug. Next in order of susceptibility were melanomas and carcinoma of the breast. Occasional cases of carcinoma of the lung, tongue, ovary, and colon responded, although for much shorter periods.

At the time the report was prepared, 25 patients were living, 34 were dead, and 1 was lost to followup. The duration of favorable effect among the patients still alive ranged from 8 weeks to 16 months.

Bone-marrow depression in varying degrees was the major side effect. It was observed rarely when the drug was given orally, and more frequently with the segmental perfusion.

Sarcolysin [(p-di-chloroethyl)-aminophenylalanine] was synthesized in 1953 by Russian scientists Dr. L. F. Larionov and his colleagues, who also demonstrated its tumor-inhibitory effects in experimental animals and patients. Dr. O. Creech, Jr., and his colleagues have used the drug in treating patients by the

isolation-perfusion technique and have reported favorable effects in melanomas. The scientific literature contains numerous other reports of pharmacological and antitumor studies of sarcolysin and compounds closely related to it.

Other names for sarcolysin are sarcolysine, merphalan, CB 3007, sarcochlorin, and DL-phenylalanine mustard. It is available for research purposes as Sarcolysin from the National Drug Company, Philadelphia.

NEW HORMONE-LIKE DRUG FOR BREAST CANCER LACKS MASCULINIZING ACTIVITY

In treating certain patients with advanced breast cancer, male sex hormones have been found capable of inducing temporary regressive changes in metastatic lesions. A clinical test of a new compound, delta-one-testololactone, showed that it produced none of the masculinizing side effects usually observed with the male hormone, testosterone, to which it is chemically related. At the same time, in this study its anticancer activity was similar to that of testosterone.

This finding was reported by Dr. Albert Segaloff, Alton Ochsner Medical Foundation, New Orleans, and his associates of the Tulane University and Louisiana State University Schools of Medicine. The study was supported by a grant from the National Cancer Institute and was a part of the program of the Co-operative Breast Group of the Cancer Chemotherapy National Service Center.

The compound was synthesized in 1953 and studied in animals by scientists of the E. R. Squibb and Sons Division of the Olin Mathieson Chemical Corporation, New York City. It is available from Squibb for research only.

Delta-one-testololactone was administered intramuscularly to 23 postmenopausal patients with progressive metastatic disease. None of them had received previous hormonal therapy. Regression of most of the lesions and increase in the size of none, as determined by roentgenograms, caliper measurements, and photographs, constituted evidence of objective response to therapy.

Objective response was observed in seven patients. Response occurred in a larger percentage of patients with local or soft tissue metastases than in those with either osseous or visceral involvement.

SCALP HAIR ROOT DAMAGE CORRELATES WITH TOXIC EFFECT OF ANTICANCER DRUG

Scientists of the General Medicine Branch have continued their study of changes in scalp hair roots of patients receiving treatment for cancer. Earlier studies showed damage to hair roots following treatment with radiation and the antifolic agent, methotrexate, and an association between the extent of damage and the dose used in therapy.

In the present study reported by Drs. Robert G. Crounse and Eugene J. Van Scott, microscopic examinations were made of growing hair roots obtained from the scalp of patients receiving a variety of chemotherapeutic agents in the clinical services of the National Cancer Institute. In 57 patients effects of the following drugs were studied: 3',5'-dichloroamethopterin, triethylene thiophosphoramide (thio-TEPA), 6-mercaptopurine, 5-fluorouracil, colcemid, actinomycin D, nitrogen mustard, cyclophosphamide, and Vincaleukoblastine.

Structural damage to hair roots occurred following the administration of the drugs. The essential effect seemed to be inhibition of hair root growth, which was promptly reversible when the drug was discontinued. A close correlation was found between hair root damage and hematologic signs of drug toxicity.

The findings led the investigators to confirm their earlier conclusion that the hair is a sensitive indicator of the toxic effects of chemotherapeutic agents and to suggest that the relatively simple procedure of examining hair roots can be useful in the clinical management of patients receiving such treatment.

STAFF SCIENTIST REPORTS METHOD OF USING VIRAL TUMORS FOR DRUG SCREENING

Dr. W. Ray Bryan, of the Laboratory of Biology, has suggested an approach to employing virus-induced animal tumors in the screening of potential anticancer drugs.

Tumors induced by viruses are desirable for screening potential anticancer agents because they derive entirely from the hosts' own tissues, thereby simulating naturally occurring tumors. Since several established viral tumor systems are now available, biologically comparable tumors can be produced in large numbers. These can be used as a basis for increasing the accuracy of measuring chemotherapeutic effects.

A large store of knowledge has emerged from several years of investigation of the biological properties of such viral tumors as the Rous sarcoma of chickens, the Friend leukemia of mice, the Shope rabbit papilloma, and a leukemia of mice recently described by Moloney. Using such information, Dr. Bryan devised several "orientation charts," with which an investigator can plan a test program of chemotherapy screening using viral tumors.

The charts show the frequency of animals developing tumors with increasing dilution of virus dose and the smallest amount of virus that produces 100 percent incidence of tumors. They also show the latent period--the time elapsing between virus inoculation and appearance of tumors.

Dr. Bryan reviewed preliminary results of studies at other laboratories using the Rous sarcoma and Friend leukemia systems to show the sensitivity of these tumors in measuring antitumor activity of test compounds. After virus inoculation, antitumor effect was measured by the extent to which a drug delayed the appearance of grossly detectable tumors. It is of interest that many of the compounds showing significant antitumor effects in both these systems are among those found to be most effective in nonviral tumor test systems and in man.

CHEMOTHERAPY PROGRAM REACHES NEW HIGH LEVEL OF CLINICAL ACTIVITY

The Cancer Chemotherapy National Service Center was established in 1955 to accelerate research and speed the introduction of promising agents into clinical trial. It has accomplished both goals. Cancer chemotherapy research now constitutes the largest and most comprehensive single approach to solution of the cancer problem; more drugs are being evaluated in the clinic today than ever before.

The clinical phase of the Service Center's program expanded at an unprecedented rate. A total of 50 new compounds was added to the 109 being evaluated a year ago for anticancer activity. Of the 159 drugs now in clinical trial, only 31 were in use prior to the establishment of the chemotherapy program almost six years ago. Scientists in more than 150 hospitals throughout the United States and Western Europe are conducting some 200 clinical studies, which to date have involved more than 16,500 patients.

Results of a clinical study sponsored by the Service Center indicated that women who received both radical surgery and chemotherapy for breast cancer were substantially less likely to have recurrent disease than were women treated only by surgery. In the study, 107 women, all of whom were considered good candidates for surgical cure, underwent radical mastectomy. Fifty-seven of the patients also received an anticancer drug, thio-TEPA, administered intravenously on the day of operation and on the first and second post-operative days. The remaining 50 received a placebo. Data collected over a two-year period indicated that 83 percent of the women who received thio-TEPA have remained free of recurrent disease, while only 54 percent of the other group were still free of cancer. Although the results of this study are preliminary, they constitute the first clear-cut evidence that adjuvant chemotherapy can markedly improve the benefits of surgery.

The animal screen--the first step in determining whether a material has anticancer activity--was broadened to help overcome one of the most formidable problems in chemotherapy research: estimating the clinical effectiveness of a drug on the basis of results of animal studies. In addition to the standard three-tumor mouse screen, two transplantable rat tumors (Walker 256 carcinosarcoma and Dunning leukemia) were being employed to assess the activity of some types of drugs, particularly alkylating agents. These two tumor systems are useful in predicting the effectiveness of anticancer agents against human leukemias and lymphomas, such as Hodgkin's disease Tissue culture was added as a screening tool for the study of materials submitted in quantities insufficient for animal testing. Compounds showing activity in tissue culture can be synthesized in larger amounts for study in mice.

Tumors in hamsters and guinea pigs may be employed for screening purposes if current investigations prove them useful. Use of these tumor systems would require substantially increased production of the animals, since chemotherapy screening would use the entire present annual production.

Exchanges of technical information are helping accelerate chemotherapy research. To expedite communication in the field, the Service Center began publication of "Cancer Chemotherapy Abstracts," a monthly compilation of abstracts of reports appearing in journals throughout the world. A companion publication, now two years old, is "Cancer Chemotherapy Reports," which disseminates current information on the status of the program.

The major part of the Service Center's program is carried out under contracts with pharmaceutical firms, research institutions, colleges, and universities. Current obligations for support of active contracts, plus funds needed to activate newly approved contracts, amount to $21,380,000.

OTHER THERAPY

FURTHER STUDIES NEEDED TO DETERMINE USEFULNESS OF RADIOACTIVE SULFUR

Results of clinical studies of the use of radioactive sulfur (S^{35}) against advanced chondrosarcoma, a cartilaginous tumor, were reported by Dr. J. Robert Andrews and his associates of the Radiation and Endocrinology Branches and the Laboratory of Pathology, and by Dr. Raymond G. Gottschalk of the Veterans Administration Center, Martinsburg, West Virginia. Previous studies had shown that S^{35} arrested cartilage growth in experimental animals, and that tracer amounts of the isotope were taken up by human chondrosarcoma.

Dr. Andrews and his associates administered one curie of S^{35} intravenously in three doses to a 42-year-old male patient. Radioactivity assays showed significantly higher concentrations of the isotope in tumor tissue than in normal rib cartilage or in skin. Autoradiographs demonstrated uptake of S^{35} by viable tumor tissue, but not by necrotic tumor cells.

Subcutaneous nodules of the tumor stopped growing for five months following injection of the S^{35}. The only side effect was temporary, but severe, hematopoiesis. Dr. Andrews concluded that the dose administered may have produced a temporary halt in growth of the chondrosarcoma.

In the study conducted by Dr. Gottschalk and his associates, three patients received doses ranging from 559 to 926 millicuries of S^{35} in the form of sodium sulfate in three to six intravenous injections. In two of the patients, the rate of growth of the chondrosarcomas appeared reduced for several months. Otherwise, the results were similar to those reported by Dr. Andrews.

These findings indicate a need for studies of additional cases of chondrosarcoma, which may have different degrees of radiosensitivity or isotope uptake, to determine whether S^{35} can regularly produce significant palliation in some cases.

NEW TECHNIQUE REVEALS STATUS OF URINE FLOW THROUGH ILEAL BLADDER

Scientists of the Surgery Branch have previously reported the successful clinical use of the ileal bladder as a means of urinary diversion in patients whose normal urinary bladder function stops as a result of disease or surgery. Use of the ileal bladder, however, creates an abnormal anatomical arrangement and makes difficult the evaluation of the upper urinary tract.

Dr. James R. Jude has now reported a method in which a series of radiographs is made of the ileal bladder following introduction of a radiopaque dye. Findings in a study of 15 patients show that failure of the ureters to fill with the dye does not necessarily imply that an anatomical obstruction is present. A fortuitous valvelike action may be present, preventing regurgitation of the dye up one or both ureters. The investigators conclude that when the cause of postoperative hydronephrosis needs to be determined, these retrograde urinary ileograms together with the usual excretory urograms provide valuable information as to the status of the ileal bladder urinary diversion.

SURVIVAL OF CANCER PATIENTS

CONNECTICUT FIGURES SHOW SURVIVAL RATE OF UTERINE CANCER PATIENTS IMPROVING

Women with cancer of the uterus have a better chance of surviving today than they had 25 years ago, and the improvement may be associated with increased use of surgery in treating this disease. This is a recent conclusion based on analysis of data on life expectancy of cancer patients in Connecticut between 1935 and 1954. Earlier diagnosis resulting from physicians' use of cytologic, or cell examination, test may also have contributed to the improvement in survival of uterine cancer patients.

Survival in ovarian cancer has not improved significantly in the last 25 years. In both ovarian and uterine cancer, the outlook for younger women is better than that for older women.

The analysis of survival experience was made by scientists of the Biometry Branch, in collaboration with investigators of the Connecticut State Department of Health. The data that were comprehensively analyzed consisted of the medical records of all cancer patients (more than 75,000) in Connecticut between 1935 and 1954. The figures on survival were compared with

those reported from a number of other treatment centers throughout the country. Because the findings in Connecticut were virtually identical with those reported elsewhere, they are considered to be representative of the entire United States.

Dr. Sidney J. Cutler reported that the results confirmed those obtained in the preliminary analysis of crude data reported in a monograph, "Cancer in Connecticut--1935-1951," which was prepared in 1955 by the Connecticut State Department of Health under a grant from the National Cancer Institute. That study showed that the ratio of cancer patients surviving five years after diagnosis had risen from 1 in 4 at the beginning of the reporting period to 1 in 3 at the end of the period. It also showed that among patients treated surgically for localized uterine cancer, the five year survival rate was nearly 90 percent.

FIVE-YEAR SURVIVAL RATES BETTER FOR WOMEN CANCER PATIENTS THAN FOR MEN

Significant findings in survival rates for lung cancer patients were reported by the End Results Evaluation Section of the Cancer Chemotherapy National Service Center to the Fourth National Cancer Conference in Minneapolis. Data for microscopically confirmed, localized lung cancer diagnosed during or after 1950 and treated by surgery, showed that five-year survival rates for 116 women and 650 men were 62 percent and 30 percent, respectively. The sex difference was approximately the same for both adenocarcinoma and squamous cell carcinoma of the lung.

Additional data, reported in Public Health Service Publication No. 789, "Survival Experience of Patients with Malignant Neoplasms," showed that five-year survival rates were significantly higher for women than those for men with cancer of the thyroid gland, tongue, and salivary gland, for skin melanomas, and for Hodgkin's disease. Furthermore, significant increases since 1951 in five-year survival were reported for five sites: large intestine, rectum, and thyroid, for both sexes, and for uterine cervix and corpus.

These findings are highlights of a study of the survival experience of 212,638 cancer patients, based on data submitted by 99 hospitals participating in the Cancer End Results Evaluation Program sponsored by the National Cancer Institute. The

goal of the program is to facilitate meaningful evaluation of end results in cancer, and particularly to compare the results of surgery and radiation with those of chemotherapy.

Data are compiled in accordance with a uniform code and submitted annually to the End Results Evaluation Section by 10 individual hospitals and the cancer registries of California, Connecticut, Massachusetts, and the Veterans Administration. Each year, follow-up data are submitted, and about 50,000 new cases are added. Thus, information is now available on about 300,000 patients. Detailed analyses of some of the data will be published in 1961 by the National Cancer Institute.

This program will form the basis of the United States contribution to an international comparison of end results at the Eighth International Cancer Conference to be held in Moscow in 1962. The international program is a collaborative effort by scientists of the National Cancer Institute and some of their colleagues from Great Britain, France, Denmark, Finland, Norway, and Russia.

LEUKEMIA RESEARCH

BONE CHANGES RESULTING FROM LEUKEMIA DESCRIBED

The bony changes resulting from acute leukemia, with direct correlation between X-ray findings and histological abnormalities, have been described in a collaborative study by National Cancer Institute and Clinical Center investigators.

Drs. Theodore F. Hilbish and his associates of the Clinical Center's Diagnostic X-Ray Department; and Louis B. Thomas, Pathologic Anatomy Branch, and Claude E. Forkner, Jr., General Medicine Branch, both of the National Cancer Institute, reported the results, which formed the basis of an exhibit that was shown at the 107th meeting of the American Medical Association.

The findings showed multiple areas of bone destruction, radiolucent bands, particularly in children, some new bone formation, and heavy leukemic infiltration. Growth lines were often demonstrated in patients experiencing periods of remission following antileukemic therapy.

STAFF SCIENTISTS REPORT STUDY OF BRAIN HEMORRHAGE IN PATIENTS WITH LEUKEMIA

Dr. Emil J. Freireich, General Medicine Branch, and his colleagues of the National Cancer Institute have reported detailed studies of fatal intracranial hemorrhage in patients with acute leukemia whose leukocyte counts rose to more than 300,000 per cu. mm. An earlier paper reported the observation of a distinctive hemorrhagic lesion largely confined to the white matter of the brain in such patients. In leukemic patients who died of intracranial hemorrhage but whose blood counts were lower than 300,000, the hemorrhage was primarily subdural or subarachnoid, as seen in non-leukemic patients with thrombocytopenia.

Intracerebral nodules of leukemic cells and stasis of leukemic cells (leukostasis) in small intracerebral vessels were found only in patients with leukocyte counts of more than 300,000. Neither leukostasis nor leukemic nodules were found in patients with leukocyte counts below 100,000 per cu. mm. The data suggest that local intravascular growth of leukemic cells results in destruction of the vessel wall and formation of a leukemic nodule. Finally, hemorrhage occurs about the nodule and coalescence of many such lesions results in the large hemorrhage seen grossly. This intracerebral leukemic lesion is the major factor responsible for fatal intracranial hemorrhage in patients with high leukocyte counts.

SPINAL FLUID EXAMINATION SHOWS ONSET OF SYNDROME OF MENINGEAL LEUKEMIA

Investigators of the General Medicine and Pathologic Anatomy Branches have continued their detailed studies of the natural history of acute leukemia, particularly neurological complications. Dr. Edward W. Moore has reported results of a postmortem study of the central nervous system of 117 patients admitted to the Clinical Center.

Twenty-three (19 percent) of these patients showed the clinical syndrome of meningeal leukemia. Any patient with acute leukemia may develop the meningeal syndrome, but it is most frequently noted among boys with acute lymphocytic leukemia.

The pathological findings showed that this syndrome is related to the leukemic infiltration of the arachnoid, particularly along the brain stem, and to internal hydrocephalus. The leukemic infiltration within the arachnoid probably interferes with the flow of cerebrospinal fluid (C.S.F.) and results in increased intracranial pressure.

The degree of leukemic infiltration of the arachnoid is reflected in the C.S.F. Dr. Moore suggests that examination of the C.S.F. should allow for early recognition and treatment, with the possible prevention of hydrocephalus and other irreversible neurological lesions.

COMPREHENSIVE STUDY OF MENINGEAL LEUKEMIA SUGGESTS TREATMENT METHOD

The work was extended by the same group of scientists to a comprehensive study of meningeal leukemia, which was observed in 25 of 150 acute leukemia patients admitted consecutively to the Clinical Center during a five-year period ending April 1, 1958.

Dr. Richard K. Shaw reported that meningeal involvement is primarily a complication of acute lymphocytic leukemia in children. The syndrome may occur at any stage of the disease: as an initial manifestation, during hematologic remission, or during an active leukemic phase. Its appearance is indicative of relapse.

A diagnosis of meningeal leukemia is confirmed by examination of the cerebrospinal fluid showing increased pressure, presence of leukemic cells, and absence of infection. The characteristic radiologic finding is spreading of the cranial sutures in children.

A literature review included in the present report shows that meningeal leukemia has become more common since the advent of chemotherapy. Only 31 patients were reported between 1878 and 1948, in contrast to 69 between 1949 and 1959. The increase is partly related to the ability of antimetabolites to prolong the lives of acute leukemia patients, particularly children. Of 21 children in the Clinical Center group, 13 developed the meningeal syndrome more than four months after onset of leukemia. It appears that the failure of antimetabolites to cross the "blood-brain barrier" may play a role in the development of the meningeal syndrome.

The investigators recommend treating meningeal leukemia first with adrenal steroids administered systemically; then, if additional therapy is indicated, with whole brain irradiation or methotrexate injected into the spinal fluid.

NEGATIVE TRIAL OF DRUG PROVIDES DATA ON COURSE OF HUMAN ACUTE LEUKEMIA

The importance of scientifically designed studies in chemotherapy was highlighted by a collaborative study reported by National Cancer Institute investigators and their colleagues of the Acute Leukemia Cooperative Group B, sponsored by the Cancer Chemotherapy National Service Center.

During a previous study of the toxicity of a new anticancer drug, 6-azauracil, Institute scientists observed objective hematologic improvement in five leukemia patients whose disease no longer responded to conventional chemotherapy (e.g.folic acid antagonists, adrenocortical steroids, or purine analogs). A comparative study was therefore designed to evaluate the therapeutic efficacy of the drug in patients with advanced refractory acute leukemia.

Dr. Emil J. Freireich, General Medicine Branch, reported that eight children and seven adults received 6-azauracil while seven children and six adults received placebo. Although partial remissions were observed for varying periods in four patients in each group, the difference between the two groups in median survival time was not significant.

These findings provided an unusual opportunity to obtain information about the natural history of advanced refractory acute leukemia. A significant proportion (30 percent) of the patients showed objective hematologic improvement even in the absence of effective specific therapy. The patients had a median survival of two months from the start of the study, and children and adults showed similar survival patterns.

The toxicity of 6-azauracil limited the clinical dose to less than 10 percent of that (on a weight basis) producing antitumor effects but not toxicity in animals. These findings provide a rationale for further studies of a derivative of 6-azauracil, azauridine, which preliminary clinical studies show is better tolerated than the parent compound.

The compound, 6-azauracil, an antimetabolite, was supplied by E. R. Squibb and Sons, New Brunswick, New Jersey. It was synthesized in 1955 and shown to have antitumor activity in animals by Dr. Arnold D. Welch and his associates of the Yale University School of Medicine. Preliminary clinical studies reported in 1957 by scientists of the National Cancer Institute and the District of Columbia General Hospital suggested that the compound had some therapeutic activity in acute leukemia.

STUDY OF HOW 6-MP ACTS INCREASES UNDERSTANDING OF DRUG-RESISTANT TUMORS

Studies of the mechanism of action of the antitumor agent, 6-mercaptopurine (6-MP), are important to an understanding of resistance to the drug and to development of more effective derivatives. Results of one such study, summarizing data on the effects of 6-MP upon nucleic acid metabolism of mouse leukemia L1210 cells in vitro, have been reported by Dr. Jack D. Davidson of the General Medicine Branch.

Drug-sensitive and drug-resistant leukemic cells were compared with respect to utilization of radioisotopic precursors for the formation of nucleic acid purines. Both lines showed similar rates of nucleic acid synthesis, but the details of the metabolism were different. The sensitive line showed much greater utilization of labeled hypoxanthine (purine precursor); utilization for adenine formation was greater than for guanine formation. Resistant cells utilized labeled glycine (amino acid precursor) to a greater extent than the sensitive ones, and the utilization for guanine formation was greater than for adenine.

6-MP, which is similar to hypoxanthine except for the substitution of a mercapto (SH) group for a hydroxyl (OH) group, inhibited the incorporation of hypoxanthine and glycine into the adenine nucleotides (moieties of nucleic acid) in the sensitive cells, but had no effect on the guanine nucleotides. These findings support the hypothesis that 6-MP is metabolized to its nucleotide, and this produces a metabolic block in the conversion of inosinic acid (hypoxanthine nucleotide) to adenylic acid (adenine nucleotide). The common pathway for these two precursors lies between inosinic acid and adenylic acid.

In the resistant cells, 6-MP inhibited profoundly the incorporation of hypoxanthine into both the adenine and guanine moieties; utilization of glycine was unaffected. These results suggest that the 6-MP block in the resistant cells is located on the pathway from free hypoxanthine to inosinic acid. This pathway is of limited capacity in the resistant cells and is incapable of producing sufficient 6-MP nucleotide to inhibit the conversion of inosinic acid to adenylic acid. Resistance is, therefore, correlated with the cell's loss of the capacity to convert free 6-MP to its nucleotide derivative, which appears to be the form responsible for antitumor activity.

DRUGS USED TO TREAT MICE WITH SYSTEMIC LEUKEMIA MAY ALSO CONFER IMMUNITY

Dr. Abraham Goldin of the Laboratory of Chemical Pharmacology has reported further results in the continuing studies of the effectiveness of dihalogenated derivatives of methotrexate in prolonging the life of mice with transplanted leukemia L1210.

The current study of immunity in mice surviving the systemic disease confirms preliminary findings that many of the long-term survivors are markedly immune to reinoculation of leukemic cells, the new inoculum showing no or very slow growth. Other data suggest that the leukemia must be sufficiently far advanced, possibly systemic, for eradication of the disease by treatment with the drugs to be accompanied by the development of immunity.

Dr. Goldin concludes that the drugs act, at least in part, by influencing the host-tumor relationship so that the mice become immune. Thus, the importance of understanding the interrelationships of the host, tumor, and drug in the chemotherapy of cancer is again emphasized.

SCIENTISTS CONTINUE STUDY OF HOST-TUMOR-DRUG FACTORS IN L1210 MOUSE LEUKEMIA

Additional results of the study of the interrelationships of host-tumor-drug factors in the L1210 mouse leukemia were reported by Dr. Goldin. The finding that mice surviving systemic leukemia L1210 following treatment with drugs showed immunity to reinoculation of drug-sensitive and drug-resistant sublines of the leukemia suggested another line of investigation. This was the possibility of utilizing the immune response to enhance the effectiveness of chemotherapy of mice bearing variant forms of the disease resistant to specific drugs or classes of drugs.

Mice were inoculated on day 0 with a subline, L1210Z, which is sensitive to therapy with antifolic drugs, and on day 7 with a subline, M46R, which is resistant to antifolic drugs. Treatment with 3',5'-dichloroamethopterin (DCM), started on day 7, when the sensitive disease had become systemic, produced a median survival time of approximately 40 days from the day of inoculation of the resistant subline.

On the other hand, the survival time of DCM-treated mice bearing only the resistant subline did not exceed 20 days, even if treatment was started on the day of implantation. Furthermore, if the mice were inoculated with both lines and treatment was started on the same day, they survived no longer than treated mice bearing only the resistant subline.

The findings showed that it was possible to prolong the survival of mice bearing an antifolic-resistant variant of leukemia L1210 and receiving treatment with DCM by prior inoculation with a drug-sensitive subline. The presence of systemic sensitive leukemia L1210 appeared to elicit in the hosts an immune response, which contributed to the therapeutic effect of DCM against the resistant variant. Further study of the relationship of drugs to immunological inhibition and enhancement is continuing to learn if it offers a basis for improved cancer therapy.

STUDIES AT THE CELLULAR LEVEL

CANCER CELLS IN BLOOD SEEN MORE FREQUENTLY IN INOPERABLE PATIENTS

Dr. John F. Potter and his colleagues in the Surgery Branch, working with scientists in the Pathologic Anatomy Branch, have reported the latest results of a continuing study of the significance of cancer cells in blood.

Blood from 376 cancer patients provided the source material for the study. Specimens of peripheral blood or venous blood draining the tumor site at the time of operation were examined by a quantitative technique described by Institute scientists about two years ago for processing human whole blood so that it can be studied microscopically for the presence of malignant cells.

Malignant cells were found in the peripheral blood in 28 percent of 285 patients and in local blood (at the tumor site) of 43 percent of 91 patients. There was a significantly greater frequency of cancer cells in both peripheral and local blood of patients with nonresectable cancers than in those in the resectable group.

SCIENTISTS CONTINUE STUDY OF EFFECTS OF RADIATION ON HUMAN CANCER CELLS

Dr. Robert E. Bases, of the Radiation Branch, has reported that the antibiotic, actinomycin D, enhanced the killing effect of X-rays on single HeLa cells (human cancer cells grown in tissue culture) exposed to the drug just before or after irradiation and during clone formation. This laboratory investigation was prompted by an interest on the part of scientists in modification of the response of mammalian cells to ionizing radiation and by the observation that actinomycin D apparently produces an enhanced response to radiation therapy in cancer patients.

In the present study, actinomycin D was not unique in producing this response, as cells in culture medium containing a large percentage of heavy water (D_2O) were also more sensitive to radiation. In fact, prolonged exposure to D_2O (14 hours) enhanced the radiation sensitivity of the cells even after D_2O was removed.

MAJORITY OF CELLS REPAIR X-RAY DAMAGE BEFORE THEY DIVIDE

Continuing his studies of the effect of radiation at the cellular level, Dr. Mortimer M. Elkind, of the Radiation Branch, has reported on studies of X-ray damage and recovery in mammalian cells carried in tissue culture.

Other studies reported in the scientific literature have suggested that X-ray damage may be cumulative. It follows, therefore, that surviving cells having accumulated a sublethal amount of damage might be more sensitive to subsequent exposure to X-ray than the parental population.

To investigate the question of the presence of heritable damage, the effect of X-ray on two lines of Chinese hamster cells propagated in tissue culture was studied. The vast majority of surviving cells did not transmit sublethal damage to their progeny, but completely repaired their accumulated damage before their first division after irradiation. Also, surviving cells could undergo repeated cycles of damage and repair with no apparent weakening of the repair process.

NUCLEIC ACID DIFFERENT IN NORMAL AND LEUKEMIC HUMAN WHITE BLOOD CELLS

Results of a study of nucleic acid (DNA) from human normal and leukemic tissues have been reported by Dr. G. di Mayorca at the Sloan-Kettering Institute for Cancer Research. DNA is of particular interest at the present time because of the concept that this important component of cells is the focal point of the origin of cancer and the target of chemotherapy.

Chemical analysis showed that DNA obtained from normal spleen and leukocytes was similar to that from leukemic leukocytes. However, analysis by anion-exchange chromatography showed that the DNA of normal spleen is somewhat different structurally from that of normal leukocytes. Furthermore, the DNA of normal leukocytes is markedly different from that of leukemic leukocytes. In turn, the DNA of leukocytes from patients with chronic granulocytic leukemia differs from that from patients with chronic lymphatic leukemia.

Previous reports in the scientific literature have shown that the DNA of human leukocytes is structurally heterogeneous. The present study extends these findings to the DNA of the leukocytes of untreated leukemia patients and the DNA of normal human spleen.

HUMAN AND OTHER SERA LETHAL TO MOUSE TUMOR CELLS STUDIED IN VITRO

Dr. Maurice Landy, Laboratory of Chemical Pharmacology, reported results of a comprehensive study showing that normal serum from human and many other species exerts a lethal effect on mouse tumor cells in vitro.

Human serum interacted in the test tube for one hour at 37° C. with Sarcoma 37 ascites tumor cells in the proportion of 1 million cells to 1 ml. of serum affected the cells so that they failed to proliferate in mice; control cells gave rise to progressive growth of tumor within 8 to 12 days. Other evidence of damage by normal human serum to S-37 cells was manifested by their loss of metabolic activity, inability to exclude dyes, and changes in their structure. Of several serum components which participate in the damaging effect on tumor cells, "normal" antibody is the specific one.

These damaging effects on S-37 tumor cells were produced by serum of 11 of 17 animal species examined.

Cells of about a dozen mouse and rat tumors were capable of absorbing the "normal" antibody from human serum. The antibody was absorbed also by lymphoid tissues of mice--spleen, lymph nodes, thymus, and bone marrow--suggesting that these tumors and lymphoid tissue possess a common antigen.

It was concluded that there is thus "widely distributed among animals a type of 'natural' antibody, which...may serve as a barrier against invasion by cells of foreign species."

TECHNIQUE IS DESCRIBED FOR DETERMINING ORIGIN OF CULTURED CELL LINES

The planned use of cultures of living cells in studies of the viral etiology of human cancer has highlighted the need for characterizing established cell lines. Currently almost 100 different kinds of cells are under cultivation in laboratories throughout the country. Under the auspices of the National Cancer Institute Viruses and Cancer Panel, a Cell Culture Collection Coordinating Committee was recently established under the

chairmanship of Dr. Jerome T. Syverton, University of Minnesota, for the purpose of characterizing, standardizing, and preserving mammalian cell lines.

Dr. Vittorio Defendi, of The Wistar Institute of Anatomy and Biology, has reported that immunological and karyological (chromosome morphology and constitution) characteristics gave sufficient information to identify 11 established cell lines in vitro. No criterion alone was sufficient, but together the criteria corroborated each other.

In the study now reported, three characteristics were found to be highly indicative of the originating species of a given cell line. These were: 1) Sensitivity to nucleoprotein antiserums, indicating antigen-antibody combination. Whether or not cells of the line under test were destroyed by the antiserums showed that the cell line was of human or murine origin. 2) Sensitivity to skin grafts from C3H mice, indicating the presence of murine transplantation antigens. Adult mice of a selected inbred strain were inoculated intraperitoneally with cells of the test line. Some days later the mice were given skin grafts from a donor of a different inbred strain. Curtailed survival of the grafts indicated the sharing of at least one important transplantation antigen between the test cells and the strain providing the skin grafts, and thus showed that the line under test was of murine origin. 3) Chromosome studies.

Of the 11 cell lines studied, four--three of stated human origin and one of stated rabbit origin--were found to be of mouse origin, probably consisting of L cells. Dr. Defendi suggested that these four cell lines represent contaminations from L cells, which appear to have exceptional ability to survive.

FOUR SIMILAR HUMAN CELL LINES DIFFERENTIATED BY RESPONSES TO CHEMICALS

Another study conducted by scientists of the Laboratory of Chemical Pharmacology was concerned with the differentiation of morphologically similar cell lines. Such cell lines are commonly characterized by nutritional requirements, chromosome counts and anomalies, sex-chromosome markers, morphologic variations, immunologic response, and virus susceptibility.

Henry C. Orr and Dr. Morris Belkin have reported the possibility of characterizing four morphologically indistinguishable cell lines by their response to certain chemicals. The strains are: HeLa, a line of malignant cells derived from a patient with uterine cancer, and three cell lines, designated as D227, D189, and D164, which resulted from spontaneous in vitro transformations of normal human fibroblasts into cells histologically similar to malignant cells.

Strain D227 was the only line to respond to Suramin sodium. After one to two days' exposure to this compound, D227 exhibited a number of changes, including fragmentation and beading of the cell membrane.

Pronounced vacuoles appeared in the cytoplasm of cells of strain D189 after three days' exposure to 0.01 mg. of hydrocortisone per ml. of nutrient medium. Exposure to a tenfold dose of hydrocortisone for ten days produced sporadic vacuolization in D164 cells and cellular enlargment and nuclear aberration in HeLa cells.

These findings demonstrate that the strains are free from cross-contamination and suggest that the D derivatives represent completely different biological variants.

TUMORS IN LABORATORY ANIMALS

CARCINOGENS FOUND MOST ACTIVE IN NEWBORN MICE

Confirmation of the extraordinary susceptibility of baby mice to carcinogenic stimuli was afforded by work by Dr. Roger W. O'Gara, Laboratory of Pathology, and Dr. Margaret G. Kelly, General Medicine Branch. They reported that microgram quantities of carcinogenic hydrocarbons introduced subcutaneously into newborn randomly bred Swiss mice produced pulmonary tumors in all of them within 16 weeks. Similar results were obtained in two inbred strains of mice with low spontaneous incidence of pulmonary tumors. The number of pulmonary tumors obtained from exposure to the carcinogen on the first day of life was substantially greater than that from the same dose injected at 1, 3, or 6 weeks of age. In addition to the pulmonary tumors, some mice developed subcutaneous fibrosarcomas. Tumors of the sebaceous glands, adrenal cortex, and liver appeared, as well as leukemias and lymphosarcomas.

SMALL AMOUNTS OF X-RAY PREVENT GROWTH OF MOUSE TUMOR CELLS AS METASTASES

In a search for more effective means of dealing with the dissemination of viable cancer cells during surgery, scientists of the Surgery Branch conducted a laboratory study of the efficacy of small amounts of radiation in decreasing the production of metastases.

Dr. Robert C. Hoye reported that amounts of radiation ranging from 170 to 2000 r produced an apparent change in the cells of 4 transplanted mouse tumors without significantly altering the growth of the tumors themselves. The cellular change prevented the growth of the majority of cells as lung metastases, when the cells were artificially disseminated from the host tumor 24 hours following radiation.

The mechanism responsible for this change in growth potential of disseminated cells is not yet known.

FIBRINOLYSIN INHIBITS PULMONARY METASTASES IN TUMOR-BEARING RATS

Dr. Carlo E. Grossi at the Sloan-Kettering Institute for Cancer Research reported that human fibrinolysin effectively inhibited the development of pulmonary metastases in a large percentage of rats bearing the Walker 256 carcinosarcoma.

Rats were injected first with fibrinolysin solution and then within a few minutes with suspensions containing large numbers of tumor cells. The animals were sacrificed when they seemed about to die or at the end of nine weeks. Gross autopsy was performed in all animals.

Only 25 percent of 113 treated animals were found to have metastatic deposits in the lungs. In contrast, 81.5 percent of 103 control animals developed one or more pulmonary metastases. The liver, spleen, and other organs appeared normal in both groups.

Dr. Grossi suggested that the existence of fibrinolytic activity at the time of inoculation of tumor cells prevented the tumor cells from lodging in the lungs. He suggested further that clumping of cells may be necessary for lodging of cells and that fibrin formation may maintain them until metastatic growth can take place.

INSTITUTE STUDY SHOWS GROWTH-STIMULATING FACTOR IN TUMOR IS SODIUM ION

Previous studies by scientists of the Laboratory of Physiology have shown that tumor-bearing rats fed a diet containing lyophilized tumor tissue consume more food and water, gain more weight, and produce larger tumors than do rats receiving casein.

Dr. Julius White reported that the growth stimulatory properties of Walker carcinosarcoma 256 tissue were shown also by the water-soluble fraction prepared from it, and that the factor principally responsible for the activity was sodium ion. Addition of sodium chloride to the water-insoluble fraction of Walker 256 tumor tissue and to the casein diet resulted in markedly increased water intake, food intake, and weight gain.

RELATED FUNDAMENTAL STUDIES

ANTIBODY ACTIVITY FOUND IN FRACTIONS ONE AND FIVE OF HUMAN GAMMA GLOBULIN

Dr. John L. Fahey, General Medicine Branch, has reported further results in his study of the nature of gamma globulins from human blood. In an earlier report, he described their isolation from the rest of the normal blood serum proteins and their fractionation by the technique of anion-exchange cullulose chromatography. Ninety to 95 percent of the gamma globulins comprised a heterogeneous group of protein molecules of about the same molecular size; these were separated into four fractions, which differed in electrical charge and carbohydrate content. A fifth fraction contained five to ten percent of the gamma globulins and although comprised largely of protein macromolecules, (giant size molecules) also included a small amount of the smaller molecules.

In the present study, the distribution of normal and abnormal physiologic activities among the five gamma globulin fractions was investigated. Antibodies to mumps virus, Histoplasma capsulatum, thyroglobulin, and typhoid H antigen were found in fraction 1. Antibodies to the rheumatoid arthritis factor and typhoid O antigen were found in fraction 5. Several activities were found in fractions 1 and 5--anti-A and anti-B isohemagglutinins, the Rh antibodies, and the anti-liver-nucleoprotein extract activity, when present in the serum of patients with lupus erythematosus. The finding of the same activity in two fractions indicated that the antibody activity was associated with distinctly different gamma globulin molecules.

Dr. Fahey reported that fractions 2,3, and 4, comprising about 20 percent of the total gamma globulins, could not be demonstrated to contain the major component of any physiologic activity.

GROWTH PATTERN STUDIED IN RATS AFTER REMOVAL OF PITUITARY GLAND

Dr. Roy Hertz, Chief of the Endocrinology Branch, has reported detailed studies on the growth pattern in young rats which were hypophysectomized (pituitary gland removed). Then multiple pituitary grafts were implanted underneath the kidney capsule in the rats from which the pituitary was removed. The grafts supported substantial somatic growth--about two-thirds the normal rate--but failed to maintain normal thyroid, adrenal, and gonadal function.

These results contribute to clarification of the relationship between hypothalamus and pituitary, and show that in the rat, production and secretion of the growth hormone are not completely dependent upon an intact hypothalamic-pituitary system. Dr. Hertz suggests that such pituitary grafts, which are known to increase the incidence of breast cancer in mice, may exert their tumor-enhancing effect at least in part by producing growth hormone. Moreover, the data indicate that the demonstrated beneficial effects of the removal of the pituitary stalk in breast cancer patients may not be mediated through suppression of growth hormone.

AGE OF PERSON AFFECTS AMOUNT OF ACID-SOLUBLE COLLAGEN IN HUMAN SKIN

Dr. William G. Banfield, Laboratory of Pathology, has reported that the amount of acetic acid-soluble collagen present in human abdominal skin varies in an orderly fashion throughout life.

Collagen is the main supportive protein of skin, connective tissue, and bone. The changes in collagen in relation to age and pathologic conditions are being investigated in order to arrive at an understanding of its formation and metabolism. An earlier study reported by Dr. Banfield showed that there is a sharp drop in the concentration of soluble collagen in human skin at one year of age.

The results of the present study show that until age 20 soluble collagen gradually decreases, from age 20 to 40 it decreases more sharply, and then rises again. At 60 to 80 years of age, 80 percent of individuals whose skin was tested showed soluble collagen. After age 80, soluble collagen declines slightly.

The author speculates that part of the change in solubility of collagen may be the result of a changing hormone pattern, reflected in the collagen metabolism. The actual change that takes place in the collagenous composition resulting in variations in solubility is not yet clear.

NEW WAY TO PREPARE SLIDES FOR ELECTRON MICROSCOPY REPORTED

Dr. Albert J. Dalton, Laboratory of Biology, has reported a simplified method of staining thin sections of biological material with lead hydroxide for electron microscope studies.

Essentially the technique consists of staining specimens with lead acetate in solution and then exposing them briefly to ammonium hydroxide vapors. Dr. Dalton found that electron micrographs taken of specimens prepared in this way were at least equal in resolution and contrast to those obtained by the more involved procedure of staining with a solution of lead hydroxide, described about two years ago in the scientific literature.

EXCHANGES OF RESEARCH INFORMATION

CANCER EXPERTS REPORT AT QUADRENNIAL MEETING OF NATIONAL CONFERENCE

The Fourth National Cancer Conference was held in September at the University of Minnesota, sponsored jointly by the National Cancer Institute and the American Cancer Society. About 1,500 scientists and physicians attending the quadrennial meeting heard reports by the nation's leading cancer authorities and experts from Great Britain, Denmark, Russia and other foreign countries.

Over 90 scientific papers were presented at general sessions which dealt with causation, development and spread, and treatment, and in symposia on the state of knowledge of cancer of specific sites and the problem of cancer control.

A feature of the conference was a special evening program, "Cancer in the World Around Us," to which the public was invited. The speakers discussed environmental factors associated with the occurrence of cancer, such as air pollution, industrial hazards, food additives, radiation, and smoking.

Highlights of the scientific reports included: statistical data on the association of smoking, alcohol consumption, childbearing, and dietary habits with the occurrence of various kinds of cancer; improved prognosis of Hodgkin's disease, with

statistics obtained in a series of 285 patients showing that 20 percent survived at least 25 years and more than a third survived at least 5 years; increased survival rates in the past 25 years for women with six relatively common forms of cancer of the reproductive and digestive systems, with an exception noted for breast cancer in which no significant improvement in survival has been noted; a 50 percent cure rate for patients with localized lung cancer treated by surgery; evidence that women who undergo surgery for localized lung cancer are twice as likely as men to live at least five years after the disease is diagnosed.

Of the thirty scientific exhibits at the conference, the National Cancer Institute co-sponsored two with the American Cancer Society: a visual presentation of the conference theme "Changing Concepts Concerning Cancer" and a display of books, journals and publications on cancer. The Institute contributed exhibits on "Virus-Cancer Research" and "International Aspects of Cancer."

The proceedings of the conference are to be published in book form by J. B. Lippincott Company.

FIVE SOVIET SCIENTISTS VISIT CANCER INSTITUTE

A Soviet delegation of five scientists engaged in cancer research made a three-day visit to the National Cancer Institute in September under an agreement between the United States and Russia for cooperation in exchanges in the scientific and cultural fields in 1960 and 1961.

The delegation headed by Dr. Nicolai Blokhin, President of the Academy of Medical Sciences, Moscow, had previously visited the Memorial Sloan-Kettering Cancer Center, New York City. The visitors talked with National Cancer Institute scientists, observed research projects, and presented some of their recent findings in tumor virus and experimental chemotherapy research at a meeting sponsored by the Institute.

NCI MONOGRAPHS PRESENT CONFERENCE PROCEEDINGS, SERIES OF RELATED PAPERS

The Journal of the National Cancer Institute has published to date four National Cancer Institute Monographs. The Monographs present the proceedings of conferences and symposia, and series of papers dealing with cancer and closely related research fields. They are for sale by the Superintendent of Documents, Government Printing Office, Washington, D.C.

The titles in the series are: No. 1, "Estrogen-Induced Tumors of the Kidney in the Syrian Hamster" (December, 1959); No. 2, "Symposium on Normal and Abnormal Differentiation and Development" (March, 1960); No. 3, "Conference on Experimental Clinical Cancer Chemotherapy" (August, 1960); and No. 4, "Symposia--Tumor Viruses" (September, 1960).

The Journal of the National Cancer Institute is distributed in 75 countries, including the U. S. S. R. Approximately 18 percent of the papers describe research findings of investigators in foreign countries. The Journal, established in 1940, was published bi-monthly for 16 years, and since July 1956 has appeared monthly.

STAFF AND PROGRAM CHANGES

DR. KENNETH M. ENDICOTT HEADS REALIGNED STAFF

Dr. Kenneth M. Endicott, formerly National Institutes of Health Associate Director for Training, became Director of the National Cancer Institute July 1. He succeeded Dr. John R. Heller who, after nearly 30 years in the Public Health Service, became president of the Memorial Sloan-Kettering Cancer Center in New York City. Dr. Heller served as Director of the Institute for 12 years.

A realignment of administrative responsibility for grant support of research and training, and for field studies, became effective in September with the appointment of two Associate Directors. Dr. Ralph G. Meader, Chief of the Research Grants Branch since 1948, was designated Associate Director for Grants and Training. Dr. Michael B. Shimkin, Chief of the Biometry Branch since 1954, was named Associate Director for Field Studies.

Dr. Stuart M. Sessoms, who has supervised the Institute's worldwide drug development program since November 1958, was also given the title of Associate Director and continues to head the Cancer Chemotherapy National Service Center.

Full responsibility for administration of intramural clinical research was given to Dr. Charles G. Zubrod. Dr. Carl G. Baker, Assistant Director, was assigned responsibility for intramural nonclinical research. Dr. G. Burroughs Mider, now Director, Laboratories and Clinics, National Institutes of Health, formerly administered both of these areas of intramural cancer research.

Dr. Eli M. Nadel, formerly Assistant to the Associate Director for Intramural Research, National Institutes of Health, was appointed an Assistant Director of the National Cancer Institute.

INSTITUTE NOW AWARDS CLINICAL TRAINEESHIPS TO EDUCATIONAL CENTERS

Under a new procedure for administration of its clinical traineeship program, the National Cancer Institute awarded 143 grants, totaling $1,059,401, to teaching hospitals and medical research centers throughout the country for advanced training of physicians in the diagnosis and treatment of cancer. The grants were awarded on recommendation of the National Advisory Cancer Council and became effective in July.

The grantee institutions selected the individuals to be trained and set their stipends according to the applicable salary scale. Stipends formerly were awarded directly to the individuals after they had been accepted by their chosen places of training.

More than 200 physicians in about 80 institutions are currently being trained under the program, which was established in 1937. Their fields of study include pathology, radiology, surgery, and internal medicine. Trainees are graduate physicians under 40 years of age who have had at least two years' prior training.

FUNDS TO BUILD RESEARCH FACILITIES ARE AVAILABLE ON NON-MATCHING BASIS

A total of $5,000,000 from the National Cancer Institute's 1961 appropriation has been allocated for grants to research institutions to pay the cost of construction of facilities for research on cancer. The program is authorized for one year only. Grantees are not required to match the awards, but their ability to supplement them is considered in reviewing applications.

Eligible institutions are those actively engaged in cancer research. Institutions making application are required to show that construction is needed for more effective cancer research by the key scientific staff already employed, that the amount needed for construction is substantial when measured against available and potential resources, that every effort has been made without success to secure matching funds, and that the cost of the proposed construction is reasonable.

Processing of the applications is similar to that for cancer research grants. They will be reviewed by staff members, consultants, and the National Advisory Cancer Council. Awards will be made by the Surgeon General of the Public Health Service with the advice of the Council.

NEW INSTITUTE LABORATORY-OFFICE BUILDING PLANNED

The National Cancer Institute began study of its program plans as related to space requirements after $700,000 was provided in the 1961 appropriation for planning a new laboratory-office building. Institute program leaders and members of the Research Facilities Planning Branch, Division of Research Services, National Institutes of Health, are conferring with scientific and administrative personnel on information needed for drawing up architectural plans. The building will contain approximately 100,000 square feet and will permit expansion and consolidation of the intramural and extramural components of the Institute's research programs.

Items of Interest on Program Developments and Research Studies

Conducted and Supported by the National Heart Institute

ATHEROSCLEROSIS AND CORONARY HEART DISEASE

MER-29 LOWERS CHOLESTEROL BY BLOCKADE OF FINAL STEP IN ITS SYNTHESIS BY LIVER

Studies by NIH scientists have pinpointed the mechanism by which MER-29 lowers serum cholesterol levels. By blocking the conversion of desmosterol to cholesterol, the drug inhibits cholesterol synthesis by the liver at the very last step of the complex sequence of reactions by which the cholesterol molecule is formed.

These studies were made by Drs. Joel Avigan and Daniel Steinberg, of the NHI Laboratory of Cellular Physiology and Metabolism; and Mr. Malcolm J. Thompson and Dr. Erich Mosettig of NIAMD. Their findings are reported in Progress Against Cardiovascular Disease (2: 525-530).

The scientists observed that the sterol material in the livers of rats treated with MER-29 had properties that differed subtly, but definitely, from those of cholesterol, the major sterol of normal livers. Careful analysis revealed the presence of large amounts of desmosterol, a compound identical to cholesterol except for two missing hydrogen atoms at one point in the molecule.

When they injected radioactive acetate, a cholesterol precursor, into animals pretreated with MER-29, they found that most of the radioactivity accumulated in desmosterol rather than in cholesterol. Clinical studies also showed that desmosterol accumulated in high concentrations in the serum of patients receiving the drug.

These and other findings indicated that MER-29 lowers serum cholesterol by inhibiting the desmosterol-cholesterol conversion. They also help to clarify the process of normal cholesterol biosynthesis in man by providing factual evidence

that desmosterol is an intermediate in this process.

In humans the most important source of serum cholesterol is not that consumed in the diet, but that synthesized by the liver, which readily manufactures cholesterol from fats, carbohydrates, or proteins--all of which can give rise to acetate, the primary building block. Thus the possibility of lowering serum cholesterol levels by the use of drugs which inhibit the body's own production of cholesterol has attracted increasing attention during the past few years. MER-29 appears to be the most promising of the compounds developed thus far.

The synthesis of MER-29 was announced in 1958 by Frank Palopoli and co-workers at the William S. Merrell Company, Cincinnati; and its cholesterol-lowering effects in rats were announced in 1959 by Thomas Blohm and Robert Mackenzie of the same company. The drug, also called "Triparanol," is marketed by the William S. Merrell Company, who also made the drug available for clinical research.

The NIH clinical studies showed that MER-29 reduced serum cholesterol levels in patients without producing any observed toxic effects. However, the fall in serum cholesterol was accompanied by a rise in serum desmosterol, so that total serum sterol levels were not greatly changed, and were actually increased in some patients. The investigators suggest that the biologic role of desmosterol warrants more thorough investigation, since information thus gained might be pertinent to the safe long-term use of drugs, such as MER-29, which might lead to the pile-up of compounds normally present in the body only in very low concentrations.

GRANTEE STUDY EVALUATES PROGNOSIS OF VICTIMS OF FIRST HEART ATTACKS

An NHI grant-aided study of survival and factors affecting survival after the acute phase of first heart attacks (myocardial infarction) has shown that more than half of the 224 patients studied survived for five years or more. The five-year outlook was brighter for younger patients than for older ones, was brighter for men than for women, and was brightest for patients able to resume normal activity after their attacks.

The prognosis of patients surviving for more than one month after their first clinically diagnosed heart attack was evaluated by Drs. J. L. Juergens, J. E. Edwards, R. W. P. Achor,and H. B. Burchell, of the Mayo Clinic, Rochester, Minnesota. The scientists determined the rates of survival for 224 patients three, five, and ten years after the initial attack. They also evaluated statistically the effects on

survival of such factors as age at onset; sex; pre-existing angina or hypertension; congestive heart failure developing subsequent to the attack; occupation (active or sedentary); and degree of physical disability resulting from the attack. Their findings are reported in the **A. M. A. Archives of Internal Medicine** (105; 444-450).

The average age at time of onset was 62 years (60 years for men, 67.5 years for women). Follow-up of these patients showed that 68.8% survived for three years or more after their attacks; 55.4% for five years or more; and 29.2% for ten years or more.

Not surprisingly, five-year survival rates were highest among those suffering attacks in their forties or fifties (78.6% and 62.5%), declined moderately among those having attacks during their sixties (to 56.6%), then more sharply among those having attacks in their seventies or eighties (to 25.9% and 20%, respectively). Also no surprise was the finding that pre-existing angina or hypertension, or congestive heart failure developing after the attack, made the five-year prospect bleaker--by about 25% on the average.

The study also indicated that five-year survival rates were not significantly affected by the degree of physical activity involved in the occupations of male patients; were considerably higher for men than for women; and highest for those patients who were able to resume normal activity after their attacks. The better prognosis enjoyed by the men could not be fully accounted for either by age differences at time of onset or by any other factors observed in the course of the study. The excellent five-year survival rate (73.2%) of patients who were able to resume normal activity after their attacks has also been observed in other studies, and appears to be the best index of a good prognosis.

Necropsy studies on 79 patients who survived their initial attacks but died later revealed that 82% subsequently died from cardiovascular disease. Of these, 34% succumbed to recurrent myocardial infarction.

HYALURONIDASE REDUCES EDEMA IN ISCHEMIC HEART MUSCLE The edematous fluid accumulations in heart muscle which follow coronary artery ligation in dogs, are markedly reduced by hyaluronidase injections, report Drs. J. Martins de Oliveira, M. N. Levy, and Mr. M. I. Schock, of St. Vincent Charity Hospital in Cleveland. Their NHI-supported studies are reported in the American Heart Journal (60: 106-109).

Dr. Martins de Oliveira and coworkers tied-off a branch of the left coronary artery in 14 dogs to produce infarction and edema of the posterior papillary muscle (PPM). Eight of these dogs received hyaluronidase intravenously immediately after ligation and at hourly intervals for 5 hours, whereas the remaining 6 dogs received placebo injections of saline solution. Six non-ligated dogs served as normal controls.

Specimens of the PPM and of anterior superior left ventricular tissue (ASLV) not affected by coronary ligation, were taken from the animals at sacrifice 5 hours after ligation. Differences in tissue water-content, measured by comparing tissue weights before and after drying the tissues in an oven, were used as a simple, accurate index of edema formation.

From these studies the scientists found that PPM water content averaged 77.48 percent in the hyaluronidase-treated group, but increased to an average of 79.92 percent in dogs that received only placebo injections. ASLV values in both of these groups, 76.39 and 76.21 percent respectively, approximated normal control values obtained from non-ligated dogs. The differences between normal and ischemic myocardium in the experimental groups are statistically significant, report the investigators.

"In the present study, hyaluronidase significantly reduced the water content of the ischemic myocardial areas, bringing it to values close to those of the normal regions," conclude the Cleveland scientists. However, they state,"The practical application of our experiments is still uncertain, since it remains to be proved whether or not it is beneficial to reduce edema in an ischemic myocardium. It may be postulated, however, that the increased tissue pressure caused by this edema may exert a compressive action upon the collateral vessels, thereby reducing still more the flow of blood to the damaged areas. Further investigations are now being carried out in an effort to answer this question."

Hyaluronidase, or "spreading factor," is a mucolytic enzyme used by physicians to promote diffusion and absorption of drugs injected subcutaneously. Recent findings by Hungarian scientists, who demonstrated that intravenous hyaluronidase produces a faster diffusion of fluids through extracellular spaces and enhances lymphatic drainage, provided the basis for using hyaluronidase in the present study.

HEART ATTACKS TREATED BY CORONARY INJECTIONS OF CLOT-DISSOLVING ENZYME

A technique using a cardiac catheter to inject the clot-dissolving enzyme fibrinolysin into the coronary arteries has been used by NHI grantees in the treatment of eight patients with myocardial infarction. The procedure, which was well tolerated by the patients, appears to possess two important advantages over oral or intravenous means of administering fibrinolytic therapy in such cases. It provides high concentrations of clot-lysing enzymes at the site of the clot without unduly elevating whole-system concentrations; and it allows intermittent perfusion, a procedure that appears to be more effective in dissolving clots than is continuous perfusion.

These studies were conducted by Drs. Robert J. Boucek and William P. Murphy, Jr., of the University of Miami School of Medicine, Miami, Florida. Their findings are reported in the American Journal of Cardiology (6: 525-533).

The technique involves passing a cardiac catheter into the aorta via the brachial artery and, under fluoroscopic guidance, positioning the catheter tip at the root of that great vessel. This brings the catheter orifice in line with the coronary branches, so that fibrinolysin injected through the catheter will be swept into these vessels with their blood supply. To assure that the fibrinolysin is delivered at the exact time that the coronaries are filling with blood, the injection is made by a small pump activated by the R wave of the electrocardiograph. Best results were obtained in this study when injections were made during every fourth cardiac cycle.

The patients selected were treated within 4-12 hours after onset of the attack. Because of its reported enhancement of clot lysis, heparin (an anticoagulant) was perfused with the fibrinolysin (as Thrombolysin; Merck, Sharp, and Dohme).

The effectiveness of therapy was evaluated on the basis of the patients' clinical response; subsequent ECG data; and determinations of serum glutamic oxaloacetic transaminase (GOT), an enzyme released from injured heart tissue and which provides an index of the extent of heart damage. No attempt was made to evaluate clot lysis by angiology because it was felt that injections of radiopaque substances, which are somewhat toxic, might be harmful to heart tissue already hyperirritable.

The evaluation, though limited by available methodology, suggested that most patients were benefited by the therapy. All had an unusually mild clinical course in the hospital. All but two had serum GOT levels considerably lower than those of unperfused controls, indicative of less heart muscle damage. GOT

levels were lowest in patients treated within four hours of the attack, highest in those treated 9-12 hours after. This indicates that fibrinolytic therapy, to be effective, must be initiated quickly, since it appeared that heart damage became irreversible about four hours after myocardial infarction.

While this study does not offer conclusive proof that fibrinolytic therapy is beneficial in the treatment of myocardial infarction, the technique used may prove extremely useful for future studies in this important, rapidly developing research area.

ANDROSTERONE LINKED TO HIGH SERUM CHOLESTEROL OF THYROID DEFICIENCY

Androsterone, an important end-product of the metabolism of certain male sex hormones (androgens), may be an important factor in the high serum cholesterol levels that usually accompany thyroid deficiency.

NHI grant-aided studies on the role of thyroid hormones in steroid metabolism have shown that androsterone levels in the blood are below normal in patients with thyroid deficiency and high in those with hyperthyroidism. The studies also showed that injections of androsterone produced a sharp and significant fall in the serum cholesterol levels of normal subjects and those with hypercholesterolemia. These findings suggest that androsterone, a metabolite of certain androgens, may be an important determiner of serum cholesterol levels, since these are usually elevated in hypothyroid patients and low in those with hyperthyroidism.

These findings are summarized in Annals of the New York Academy of Sciences (86: 605-611) by Drs. T. F. Gallagher, Leon Hellman, H. L. Bradlow, B. Zumoff, and D. K. Fukushima, of the Sloan-Kettering Institute for Cancer Research, New York.

Androsterone is one of two major end-products from the metabolism of testosterone and certain other androgens; the other, etiocholanone, is structurally similar but differs sharply from androsterone in its biological activities. The scientists found that, in euthyroid patients (normal thyroid function), the ratio of androsterone to etiocholanone was 40:60. However, the androsterone fraction was only 15% or even less in patients with hypothyroidism from myxedema; conversely, it was 50% or higher in those with hyperthyroidism from Graves' disease, toxic nodular goiter, or toxic adenoma.

The scientists found that the androsterone fraction was sharply increased in euthyroid patients when they were given thyroid hormone, and was decreased toward normal in patients whose hyperthyroidism was corrected by therapy.

The cholesterol-lowering effects of androsterone might seem paradoxical, since the androgens from which it is derived are normally associated with high serum lipid levels. However, the scientists point out, hormone metabolites are not necessarily inactive end-products of a spent hormone. They may have their own important physiological functions that are not related in any way to those of the substances from which they are derived.

These findings are the basis for an hypothesis, advanced by the scientists, that the recognizable effects of thyroid excess or deficiency are mediated by the availability of androsterone in the body. They also suggest that androsterone deficiency may be one of the mechanisms involved in the development of hypercholesterolemia and, perhaps, of atherosclerosis.

NEW TECHNIQUE PROVIDES MORE SENSITIVE MEASURE OF PLASMA CLOT LYSIS

Using a sensitive, direct assay technique that closely approximates in vivo conditions, NHI grantees have measured the degree of clot-dissolving activity in human plasma. They report in the Journal of Clinical Investigation (39: 426-434) that such activity depends primarily upon an activator whose concentration in plasma varies widely in response to stress, certain drugs, and disease.

For these studies Drs. W. D. Sawyer, A. P. Fletcher, Norma Alkajaersig, and Sol Sherry, of the Washington University School of Medicine, St. Louis, prepared clots from aged human plasma enriched with plasminogen and containing traces of bovine fibrinogen tagged with radioactive iodine. The methods used produced I^{131} labeled clots that were largely free of entrapped human plasma.

The clots were then incubated for two hours at body temperature in samples of unaltered plasma from healthy adults who had been subjected to stress or pretreated with certain drugs, and from patients with various chronic or infectious diseases. The degree of clot lysis was determined by measuring the amount of radioactivity released into each plasma sample as the clot dissolved.

Thrombolysis is a complex process involving many factors whose exact roles have not yet been clarified. The major components appear to be plasmin, the enzyme that actually dissolves the clot; plasminogen, its inactive precursor; and plasminogen activator, which converts plasminogen to plasmin. The circulating blood is thought to contain inhibitors for both active

principles so that, under normal circumstances, they are present in the circulation in their "neutral" forms.

A blood clot also contains plasminogen, which was trapped in the clot during its formation. In fibrinolysis, it is thought, plasminogen activator accumulates on the fibrin surface of the clot and proceeds to convert the trapped plasminogen to plasmin. The plasmin dissolves the clot from within by digesting its fibrin.

Further knowledge of this property of plasma and better means of measuring it have been made essential by recent developments in the field of fibrinolytic drugs. However, techniques heretofore available were indirect, were not sufficiently sensitive, or disturbed the complex balance between individual plasma components. The technique used in this study appears to avoid these limitations; and, with it the scientists have been able to provide experimental confirmation of concepts that formerly were largely inferential.

They found that plasma clot-lysing activity was sharply increased by sustained vigorous exercise and was increased by emotional stress or injections of nicotinic acid (a B-vitamin) or a fever-producing drug. It was also generally elevated in patients with acute infections, liver disease, or leukemia.

Conversely, clot-lysing activity was commonly observed to be below normal in patients with cancer other than leukemia, congestive heart failure, or atherosclerosis. This subnormal activity may be causally related to the thrombo-embolic complications that frequently attend these diseases.

Plasma clot-lysing activity was found to be due primarily to the plasminogen activator it contained, and upon the plasminogen in the clot rather than that in the plasma. Clot lysis was greatly reduced when clots containing little plasminogen were incubated in the plasma samples, and was practically nil when plasminogen-free clots were used. Clot lysis was also reduced by the addition of E-aminocaproic acid, which in low concentrations effectively inhibits plasminogen activator, but not plasmin.

CLOT-DISSOLVING ENZYME ISOLATED FROM THROMBIN BY NHI GRANTEES

Chemical "dissection" of thrombin has yielded a new clot-dissolving, or fibrinolytic enzyme identified as thrombin-E, report Drs. Walter H. Seegers, Ricardo H. Landaburu, and J. Frederic Johnson at Wayne State University

in Detroit. The scientists observed powerful fibrinolytic effects of the new enzyme following its intravenous infusion in dogs.

Their findings published in Science (131: 726) stem from an earlier observation that thrombin, long known for its important physiological role in catalyzing the formation of blood clots, has both the power to form and subsequently dissolve fibrin clots under certain laboratory conditions.

In their currently reported studies, the investigators attribute the lytic activity of thrombin to a distinctly different enzyme which they named esterase thrombin, or thrombin-E, to distinguish it from thrombin-C, the parent compound.

They produced thrombin-E in pure form by acetylating thrombin-C with acetic anhydride. This destroyed the clotting power of thrombin-C and almost doubled the activity of thrombin-E, which was then stabilized by drying.

Dog experiments demonstrated the ability of intravenously infused thrombin-E to dissolve blood clots. In a typical experiment, 15 mg. of dried thrombin-E dissolved in 100 ml. of physiological saline solution, was slowly injected over a period of 1 hour and blood samples repeatedly withdrawn. Dr. Seegers and co-workers report that at the end of the infusion period, blood samples clotted but subsequently lysed within an hour. "Occasionally the lytic phenomenon was so pronounced that the blood would not clot spontaneously upon withdrawal or even after thrombin was added," they stated. The dogs tolerated the procedure very well; the few physiological effects observed, such as lowered platelet and white cell counts, increased blood sugar levels, and lytic phenomena, disappeared within 24 hours.

The investigators speculate that thrombin-E may function by activating physiological processes concerned with fibrinolysis and thus the maintenance of blood fluidity. "This opens possibilities for the useful application of thrombin-E," they said.

ARACHIDONIC ACID POSSIBLE FACTOR IN RESISTANCE TO ATHEROSCLEROSIS

Studies by NHI grantees suggest that arachidonic acid--a highly unsaturated fatty acid found in certain fish oils, dairy products, and fowl --may be a significant factor in the relative immunity to atherosclerosis enjoyed by animals of certain species. In these studies the cholesterol fractions of serum from man and from seven other species were analyzed and compared. The arachidonic acid content of serum cholesterol esters (which

comprise about 70% of the total blood cholesterol) was found to be highest in those species most resistant to atherosclerosis, lowest in the most susceptible species.

The largest amounts of arachidonic acid were found in the cholesterol ester fractions of the rat (50%) and the dog (17%), both highly resistant species. Much smaller amounts (1.1-5.8%) were found in the cholesterol esters of the goose, chicken, rabbit, guinea pig, and pig. Severe atherosclerosis can be produced in these species by feeding them high-cholesterol diets for only a short period, even though they seldom appear to develop the disease spontaneously.

Man (7.5%) is susceptible to atherosclerosis, but usually develops severe lesions only after many years on a diet high in fat and cholesterol (although much lower in cholesterol than the atherogenic diets fed laboratory animals).

However, the scientists point out, these findings, though in accord with an hypothesis that arachidonic acid deficiency is an important causal factor in atherosclerosis, have not proved that arachidonic acid is the protective factor nor that more of it in the diet of humans will make them less susceptible.

But the possibility remains that the arachidonic acid content of human lipids may be increased by diets higher in this fatty acid or the elements from which it is synthesized (linoleic acid plus the co-factor vitamin B6). It would then remain to be seen whether such an increase would confer upon man greater immunity to atherosclerosis.

These studies were done by Dr. Leon Swell, of the Veterans' Administration Center, Martinsburg, West Virginia; and Drs. Henry Field, Jr., and C. R. Treadwell, of George Washington University. Their findings are reported in Proceedings of the Society for Experimental Biology and Medicine (104: 325-328).

FIND SERUM CHOLESTEROL NOT RELATED TO OBESITY IN HEALTHY YOUNG MEN

No significant correlation was found between serum cholesterol level and relative obesity in 159 male medical students at Johns Hopkins University, report Drs. Caroline Bedell Thomas, Johns Hopkins University School of Medicine, Baltimore, and Stanley M. Garn, Fels Research Institute, Yellow Springs, Ohio. Findings of their NHI grant-aided study appear in the journal Science (131: 42).

Obesity measured indirectly was expressed in the study as actual weight or weight in relation to skeletal chest-frame diameter as seen in chest X-ray films.

Measurements of the fat-plus-skin thickness at the level of the tenth rib was also used by the investigators as a more direct method of determining obesity. The fatty layers were seen as shadows on the X-ray films and ranged in thickness from 2 to 15 mm., averaging 7.4 mm. thick.

Serum cholesterol values varied in the students from 140 to 386 mg. per 100 ml. of serum and averaged 225 mg./100 ml.

The study revealed an insignificant correlation ("not significantly different from zero at the 5-percent level of confidence") between serum cholesterol levels and direct and indirect obesity measurements.

"The lack of relationship between the amount of body fat and the serum cholesterol level demonstrated in the present study supports the view that the nutritional status of healthy young men such as medical students is considerably less important in determining the cholesterol level than other biologic factors," conclude the investigators.

Several of these "biologic factors", which include age, sex, race, heredity, endocrine patterns, habits of smoking and exercise, and emotional stress, have been previously reported by Dr. Thomas and co-workers to bear a positive relationship to high serum cholesterol levels among the Johns Hopkins medical students.

FIND MUCOPOLYSACCHARIDES NOT CAUSALLY RELATED TO ATHEROSCLEROTIC LESIONS

Because mucopolysaccharides are often found in increased amounts at the site of atherosclerotic lesions, they have been suspected of being a causative factor in atheroma development. However, studies of the aortas of cholesterol-fed rabbits suggest that the increased amounts of mucopolysaccharides usually found in arterial tissue at the site of atherosclerotic lesions are secondary to fat deposition rather than a cause of it. The studies, supported in part by an NHI grant, were carried out by Drs. A. J. Bollet, Wayne State University College of Medicine, Detroit; Chuni Wang, Detroit Receiving Hospital; and David Adlersberg, Mount Sinai Hospital, New York. Their findings are reported in Circulation Research (8: 88-92)

Mucopolysaccharides are found in the connective tissues which bind together and support the cellular components of blood vessels and many other organs. The supportive function is accomplished by the protein collagen, whose insoluble fibers are embedded in a soluble matrix called the ground substance. Mucopolysaccharides are important constituents of the ground substance.

In recent years studies on the possible role of the arterial ground substance in atherosclerosis have shown that the development of atherosclerotic lesions appears to be accompanied by increased concentrations of mucopolysaccharides in the affected tissues. Animal studies also showed that cortisone (which retards mucopolysaccharide metabolism) slowed the development of atherosclerotic lesions while the enzyme hyaluronidase (which breaks down the mucopolysaccharide hyaluronic acid) counteracted the effects of cortisone. These findings suggested that changes in mucopolysaccharides are important in atheroma formation and might even be a causative factor.

To investigate this possibility, the scientists induced atherosclerosis in rabbits by feeding them cholesterol for 1-7 months. Animals were sacrificed at two-week intervals after the first month and their aortas analyzed both for lipids and for mucopolysaccharides. Early atherosclerotic changes were found in the aortas of animals after 1-2 months of cholesterol feeding, and moderate to severe lesions in animals fed cholesterol for three months or more. However, chemical analysis of the mucopolysaccharide content of these vessels showed no significant increases except in the more advanced lesions produced by six months or more of cholesterol feeding.

The findings suggest that alterations in mucopolysaccharides are indeed related to lipid changes in the development of atherosclerotic lesions, but are secondary to lipid deposition, not a cause of it.

NHI FINDINGS SUPPORT FILTRATION THEORY OF ATHEROSCLEROSIS

Evidence supporting the filtration theory of atherosclerosis has been obtained from studies on experimental atherosclerosis conducted by Dr. Leroy Duncan, Mrs. Katherin Buck, and Mr. Almorris Lynch, of the NHI Laboratory of General Medicine and Experimental Therapeutics.

The filtration theory assigns the chief role in the development of atherosclerosis to lipoproteins, fat-protein complexes which serve as the major vehicles of lipid transport in the body. According to this theory, intact lipoproteins carrying cholesterol pass from the plasma into the inner layer of the arterial wall. Here, because of the structure of the wall, they are trapped and give rise to atheromatous lesions.

The studies on experimental atherosclerosis were based on information gained in previous studies on the movement of plasma albumin into arterial walls. These studies had disclosed that albumin enters the aorta with a gradient of rates, entering most rapidly in the upper aorta and progressively

more slowly down its length. The investigators reasoned that plasma albumin and plasma lipoprotein might enter aortic wall by the same mechanism and thus show the same gradient of rates. When combined with the filtration theory, this reasoning led to the prediction that there should also be a gradient in the deposition of cholesterol along the length of the aorta early in the development of experimental atherosclerosis.

Subsequent determinations of the cholesterol concentrations along the length of aortas in dogs confirmed the prediction. Early in experimental atherosclerosis there is a striking gradient of cholesterol deposition, and this gradient is very similar to the gradient of rates with which albumin enters the aortic wall. The experimental demonstration of this consequence of the filtration theory offers strong support for that theory.

The scientists found that the gradient disappears later in the development of experimental atherosclerosis, and that the cholesterol concentrations eventually become higher in the lower portion of the aorta. This sequence of events suggests that the most severe atheromatous lesions tend to localize eventually in the lower aorta because of the extremely slow removal of cholesterol from that segment.

These studies, reported in Circulation Research (8: 1023-1027), also emphasize that the interplay of the rates of deposition and removal of cholesterol from the arterial wall are of prime importance in the development of atherosclerosis. Studies on factors influencing these rates are in progress.

BLOOD PRESSURE

NEW HYPOTENSIVE DRUGS MINIMIZE SIDE EFFECTS BY SELECTIVE ACTION

Syrosingopine and guanethidine, two new hypotensive drugs synthesized and marketed by CIBA, have been found to be effective in lowering blood pressure and also specific enough in their modes of action to minimize undesirable side effects. Scientists of the NHI Laboratory of Chemical Pharmacology have found that both drugs lower blood pressure by depleting the peripheral sympathetic nerve endings of norepinephrine, thereby blocking the transmission of nerve impulses that trigger blood vessel constriction.

Drugs that lower blood pressure by peripheral sympathetic blockade are not new; some have been in use for years. Most of these work by inhibiting the action of norepinephrine, an amine that is released from sympathetic nerve terminals to transmit their impulses to the muscles of the arterial wall. Reserpine was the first drug found to lower blood pressure by depleting this amine at sympathetic terminals; but only slightly larger doses may also produce undesired sedation and parasympathetic effects by depleting brain amines, and diarrhea by releasing serotonin in the intestines. A major problem in treating hypertensives with reserpine has been the maintenance of dosage schedules that would hold blood pressure down without causing these side effects.

Syrosingopine, a compound made by "tailoring" the reserpine molecule slightly, resembles reserpine in its effects on blood pressure, but is more selective in its site of action. In experiments on animals, Drs. Barbara H. Orlans, Kenneth F. Finger, and Bernard B. Brodie compared the effects of reserpine and syrosingopine on peripheral norepinephrine and on brain amine levels. Both drugs depleted peripheral norepinephrine in doses that did not affect brain amines; however, reserpine exhibited this selectivity only over a narrow dosage range, whereas syrosingopine did not affect brain amines over a wide range. This more selective peripheral action of syrosingopine is a highly desirable clinical feature that provides a greater margin of safety against undesirable side effects.

Guanethidine, a synthetic drug structurally different from reserpine, is also far more specific in its action than is reserpine. The drug depletes the peripheral sympathetic nerves of norepinephrine, but does not affect brain amine levels (due in part to its limited ability to penetrate the blood-brain barrier), therefore does not produce sedation. It does not release serotonin from peripheral body tissues, thus does not cause diarrhea. The drug is also well suited to oral

administration because it is stable in the gut and is more readily absorbed than are ganglionic blocking agents.

The work by Drs. Rosemary Cass, Ronald Kuntzman, and Bernard B. Brodie did not disclose the precise mechanism by which guanethidine depletes peripheral norepinephrine, even though the results indicate that it does so by a mechanism different from that of reserpine. There are two possibilities, both under study: the drug may impair storage of the amine or block its synthesis.

The findings on the two drugs are reported, respectively, in the Journal of Pharmacology and Experimental Therapeutics (128: 131-139) and in Proceedings of the Society for Experimental Biology and Medicine (103: 871-872).

NEUROLOGICAL DISTURBANCES IN CATRON-TREATED PATIENTS DUE TO COMMON AMINO ACID

Tryptophan is an essential amino acid found in many dietary staples, and which, under normal circumstances, produces no untoward effects on the consumer. However, when given to patients receiving monoamine oxidase (MAO) inhibitors, tryptophan is capable of producing pronounced neurological disturbances, some of them strikingly similar to those of alcoholic intoxication.

These disturbances were observed by Drs. John A. Oates and Albert Sjoerdsma, of the NHI Laboratory of General Medicine and Experimental Therapeutics, after giving tryptophan orally to seven hypertensive patients being treated with Catron, a potent MAO inhibitor. The doses of tryptophan producing these effects were only 1½ - 3 times the usual dietary intake of this amino acid. This small difference raises the question of whether diets unusually high in this or other amino acids might not modify the clinical effects and side effects of MAO inhibitors in the treatment of hypertension and especially in the treatment of certain depressed states.

In three of these patients, tryptophan produced irregularities of coordination, euphoria, release of inhibitions, slurred speech, and other effects strikingly similar to those of alcoholic intoxication. These and other patients also showed signs of drowsiness, a sharp contrast to the psychic energizing effects usually produced by MAO inhibitors. Four patients receiving the largest doses of tryptophan also exhibited exaggerated reflexes, confined chiefly to the lower extremities, and accompanied by spasms of the ankle.

The fact that tryptophan produced these effects only in patients receiving MAO inhibitors suggests that the effects might be due to one or more of the amines produced from this

amino acid. The most likely suspects are tryptamine and serotonin.

Animal studies have shown that doses of tryptophan increase the levels of both of these amines in the brain, and that this increase is augmented by MAO inhibitors. These inhibit the destruction of tryptamine and serotonin by the enzyme monoamine oxidase at the same time that tryptophan is leading to stepped-up amine production. Increased levels of either or both amines are accompanied by neurological effects that vary according to the species of animal.

In man the evidence is less direct, but tryptophan has been shown to increase urinary excretion of tryptamine and an end-product of serotonin metabolism 5-hydroxyindole acetic acid (5-HIAA). Thus it is reasonable to assume that levels of both amines in the nervous system are increased by tryptophan and MAO inhibitors, and that either or both amines may be responsible for the observed neurological effects.

These findings have been accepted for publication in Neurology.

CHRONIC RENAL HYPERTENSION NOT DEPENDENT ON PRESSOR SUBSTANCES FROM KIDNEY

Animal studies on the role of the kidney in hypertension have provided evidence that the chronic elevation of blood pressure that persists in renal hypertension is not dependent on increased blood levels of artery-constricting substances from the kidney. These studies, supported in part by an NHI grant, are reported in the American Journal of Physiology (198: 1148-1152) by Drs. Pedro Blaquier, David F. Bohr, and Sibley W. Hoobler, of the University of Michigan.

The kidney is known to be the villain in several forms of secondary hypertension and has also been suspected of playing a role in the development and/or maintenance of essential hypertension. It has been demonstrated that the kidney releases renin as an acute response to an inadequate renal blood supply; and that renin, in turn, reacts with a serum protein to form angiotensin, a potent artery constrictor. But whether this response is transient or is involved in the maintenance of chronic hypertension has been the subject of much conflicting evidence.

In the study of this problem, the scientists used a cross-circulation technique in which the femoral arteries and veins of rats with surgically produced renal hypertension were connected by plastic tubing to those of normotensive rats. Preliminary work had shown that 1) cross circulation between pairs of normotensive rats produced no significant changes in

blood pressure, and 2) elevated blood pressure produced in one of the pair by infusions of renin or angiotensin was followed shortly by elevated blood pressure in the other. Thus, if the hypertension in the surgically treated rat were due to circulating vasoconstrictors, cross circulation should also produce hypertension in the normotensive rat of each pair.

To rule out blood pressure aberrations that might be due to blood volume changes, normal and stable blood volumes were maintained by equalizing the flow rates between the animals of each pair by adjustable clamps on the plastic tubing. The animals were arranged on the two pans of a sensitive balance so that any volume variations would be promptly noted and corrected.

In one series of experiments the scientists used seven rats whose renal arteries had previously been clamped for four hours. When the clamps were removed, hypertension was produced within minutes, presumably by renin from the ischemic kidney. Cross circulation, immediately initiated between these rats and seven normotensives, resulted in elevated blood pressure in all normotensive rats. However, cross circulation between normotensive rats and those with hypertension of more than one week's duration had no significant effect on the blood pressure of the normotensives.

Thus, it appears from these experiments that circulating artery-constricting substances such as renin may play some role in the initiation of renal hypertension, but may not contribute to its maintenance.

DIET-INDUCED HYPERTENSION IN RATS SIMILAR TO HUMAN ESSENTIAL HYPERTENSION

Studies on rats made hypertensive by diets deficient in potassium or choline during early life suggest that this dietary technique may yield laboratory animals more suitable for research on the role of the kidney in essential hypertension than do commonly used methods involving renal artery constriction or removal of one kidney. Renal function studies on rats showed that the diet-induced hypertension in these animals, like essential hypertension in humans, was not the result of an inadequate renal blood supply nor was it associated with severe excretory insufficiency.

In these experiments, Drs. Fred N. White, M. P. Sambi, and Arthur Grollman, of the University of Texas Southwestern Medical Center, studied renal function in 31 rats: 10 normal controls and 21 with diet-induced chronic hypertension. The NHI grantees compared data from the two groups to determine the

effects of the experimental hypertension on rates of renal blood flow, plasma filtration by the kidney glomeruli, and excretion of urea.

Their findings, reported in the American Journal of Physiology (198 (2), 221-222, 1960), revealed significant changes from normal only in the glomerular filtration rates, which were somewhat reduced in the hypertensive rats. The findings suggest certain striking similarities, with respect to renal function, between diet-induced experimental hypertension and the essential hypertension that develops spontaneously in humans. Neither is the primary result of an inadequate renal blood supply and neither is accompanied by severe impairment of excretory capacity.

These similarities suggest that the dietary technique might yield laboratory animals especially suitable for research on kidney function and hemodynamics in hypertension, especially on more subtle renal changes that might be masked by the severe lesions and renal ischemia usually resulting from drastic surgical procedures. Furthermore the dietary procedure is simpler and less subject to failure than are most surgical techniques. The potassium or choline deficient diets need to be fed to the animals for only a short period (usually 1-5 weeks) early in their lives to produce hypertension, after which the disease is self-maintaining and progressive.

Previous studies by Drs. Grollman and White, who developed the technique, indicate that the morphological changes induced in the heart, blood vessels, and kidneys by diet-induced hypertension are also similar to those of essential hypertension in humans. Thus the technique may aid in the accumulation of experimental data that might establish relationships between experimental hypertension in animals and essential hypertension in man. Such relationships might help to clarify many of the puzzling features of this enigmatic disease.

SURGERY

CARDIAC SHUNTS DETECTED BY HEART INJECTIONS OF KRYPTON-85

Studies by NHI scientists on 48 patients have shown that radioactive krypton, injected directly into the heart via a cardiac catheter, provides a simple, rapid, and accurate means of detecting and localizing cardiac shunts. The small quantities of the isotope needed for the procedure and its short biological half-life make the method safe both for the patient and for laboratory personnel.

Shunts result from holes in the partition separating the right heart from the left heart. The right heart receives oxygen-poor blood from the body and pumps it to the lungs; the left heart receives the freshly oxygenated blood from the lungs and pumps it to the body. By allowing the mixing of oxygen-poor blood with oxygenated blood in the heart, shunts can seriously impair the efficiency of the heart in delivering oxygen to the tissues.

To detect left-to-right shunts, a catheter is positioned in the left heart and 3-5 ml. of saline containing dissolved krypton-85 injected. If no shunt exist, the gas will be pumped throughout the system and largely dissipated before making a much-delayed appearance in the lungs via the right heart. But a left-to-right shunt will allow some of the gas to cross to the right heart, where it is pumped directly to the lungs. Thus, shortly after injection the gas will appear in high concentration in the air exhaled by the patient. The radiation of the expired air is monitored by a count-rate meter and recorded continuously on a direct-writing oscillograph.

To detect right-to-left shunts, the krypton-85 is injected into the right heart and arterial blood samples drawn at a constant rate for the next 15 seconds. In the absence of a shunt, all of the gas is pumped to the lungs where about 95% of it is eliminated in the expired air before entering the arterial circulation. Thus high concentrations of the isotope in the arterial samples mean that the gas bypassed the lungs by crossing directly to the left heart.

Repeated injections of krypton-85 with the catheter tip stationed at different points makes it possible to localize shunts with a considerable degree of accuracy. The method also permits estimation of the magnitude of right-to-left shunts by relatively simple calculations.

In every patient in which this technique indicated the presence or absence of a shunt, its accuracy was subsequently verified by findings at operation or autopsy, or by the use of other

established techniques for diagnosing shunts. These included selective angiography, dye dilution curves, and krypton-85 or nitrous oxide inhalation tests. The method proved to be convenient, simple to apply during heart catheterization, and sensitive enough to permit the detection and localization of even very small shunts.

These clinical studies were conducted by Drs. R. T. L. Long, Eugene Braunwald, and A. G. Morrow, of the NHI Surgery Branch. Their findings are reported in Circulation (8: 1126-1133).

SUPERIORITY OF BUTANOL CARDIOPLEGIA REVEALED BY ANIMAL STUDIES

The frequently observed depression of heart action in patients following elective cardiac arrest has stimulated the quest for better methods of stilling the heart. Chemical methods (aortic injections of potassium citrate or acetylcholine) have been largely abandoned in favor of the anoxic method which involves clamping the aorta to restrict bloodflow to the heart. Now evidence that butanol-induced cardioplegia has certain advantages over the anoxic method is reported in the Transactions of the American Society for Artificial Internal Organs (6: 323-337) by Drs. H. H. McGuire, Jr., L. H. Bosher, Jr., and R. W. Ramsey, of the Medical College of Virginia, Richmond. Their studies were supported by a National Heart Institute grant.

The scientists divided 21 dogs into four groups according to type and duration of cardioplegia: butanol arrest of 15 or 30 minutes duration, and 15 or 30 minutes anoxic arrest. Control ventricular function data was obtained for each group prior to arrest for comparison with similar data obtained 15 and 30 minutes after cessation of arrest.

Comparison of the post-arrest ventricular function curves showed that recovery following 15-minute arrest was much more rapid in the butanol-treated animals than in the anoxic arrest group. At 15 minutes post arrest, for example, ventricular function had returned to 85% of normal in the butanol-arrested animals, but to only 49% of normal in the others. Recovery after 30-minute butanol arrest was less satisfactory, but was still much more rapid than that after 30-minute anoxic arrest.

Although the protection conferred by butanol appears to diminish if cardioplegia is maintained for longer than 15 minutes, the scientists feel that this protection might be extended for longer periods by the use of hypothermia or auxillary metabolic or cardioplegic agents.

NEW CATHETER DESIGN PERMITS SELECTIVE VISUALIZATION OF CORONARY ARTERIES

A new coronary arteriography procedure, involving the use of a special loop-end catheter, is reported in The New England Journal of Medicine (262: 325-333) by NHI grant-aided scientists in Boston. Their studies in dogs and humans show that the technique permits safe, selective X-ray visualization of the coronaries with smaller amounts of radiopaque dye than is usually required by other procedures. Further application of this technique may improve X-ray diagnosis of coronary artery disease.

The Boston scientists are: Drs. Sven Bellman, H. A. Frank, P. B. Lambert, David Littmann, J. H. Hall, and J. A. Williams of Beth Israel Hospital, West Roxbury Veterans Administration Hospital, and Harvard Medical School.

In coronary arteriography, a radiopaque substance is injected through a catheter into the base of the aorta where the coronary arteries branch out into the heart. As contrast substance flows into the coronaries, rapid-sequence X-ray pictures are taken to reveal the extent and location of atherosclerotic deposits. Success of the procedure depends upon an adequate flow of contrast medium into the coronaries, and has been accomplished in the past by either injecting large quantities of medium or by diverting more contrast material into the coronaries through aortic occlusion or temporary heart arrest. While usually safe and reliable, adverse reactions have been observed in some patients receiving large dye injections.

The new technique employs a different principle of injection whereby dye is injected through holes in the catheter-loop and directed toward the coronary openings, thus eliminating the necessity for large amounts of dye. The loop-end catheter was developed by the scientists after their dog studies had shown that ordinary, straight-tipped catheters recoil away from the coronary openings as dye is injected.

The catheter-loop is held straight by a wire guide while being inserted into the aorta and regains its circular shape upon withdrawal of the guide. The synchronous injection of dye and rapid-sequence exposure of X-ray plates may be repeated for a second set of films.

The Boston workers report that coronary arteriography with the loop-end catheter was well tolerated by both dogs and humans, and that good coronary visualization was regularly obtained. They expressed the belief that this method is inherently safer than other methods currently available, and concluded,"This approach to the problems of angiographic diagnosis of coronary artery disease shows considerable promise."

The loop-end catheter method is one of several recent developments in coronary arteriography and illustrates progress being made in this field.

TECHNIQUE INCREASES SAFETY OF SURGERY TO UNBLOCK SMALL ARTERIES

A new technique that permits surgical removal of atherosclerotic deposits, clots, or other lesions obstructing blood flow in small arteries has been developed by Drs. E. S. Crawford, A. C. Beall, P. R. Ellis, Jr., and M. E. DeBakey of the Baylor University College of Medicine. The new method overcomes the problem formerly posed by the constriction of the inner diameter of the artery at the site of the incision during the healing process. This is done by inserting a knitted dacron patch at the incision site (following removal of the arterial obstruction), then suturing the wound edges to this patch rather than to one another.

After being tested on laboratory animals, the technique was used in 93 patients suffering from lesions impeding arterial blood flow. At the site of arterial blockade a longitudinal incision was made through the artery wall, cutting through all of the diseased portion plus a short undiseased arterial segment at either end. A piece of plastic tubing was inserted to detour blood around the site while the obstruction was removed. The dacron patch was then inserted and the wound edges sutured to its circumference, the plastic tubing "detour" being withdrawn just before the final suture was drawn taut.

None of these patients developed recurrent arterial obstruction during their convalescence, and only two had recurrences during the next 30 months. This is in sharp contrast to the results of animal studies in which incisions, made in arteries of comparable diameter, were repaired by suturing the wound edges together. Every artery repaired in this way subsequently became constricted.

Endarterectomy, the direct surgical removal of lesions obstructing blood flow, has been used successfully to restore normal circulation in such large arteries as the aorta and iliac. Theoretically, the same technique should be even better suited for obstructed small arteries, such as the coronary, renal, or vertebral arteries, since lesions tend to be confined to short segments of such vessels. However, its application had previously been sharply limited by the constriction that often developed in such vessels after surgical repair--a factor of great importance in small vessels because of the attendant danger of clot formation.

By overcoming this problem, the new technique greatly extends the use of surgery for the correction or improvement of arterial insufficiency resulting from atherosclerosis or other cardiovascular diseases. It may also make possible the safe and effective use of surgery in the treatment of coronary heart disease, heretofore attended by high risk and low probability of success.

This work, which was supported in part by an NHI grant, is reported in Surgical Forum (10: 671-675).

COMBAT HEART ARREST BY MASSAGE THROUGH UNOPENED CHEST

A new method of closed-chest cardiac massage which may be used by "anyone, anywhere" to restore blood flow in patients with cardiac arrest, is described in The Journal of the American Medical Association (173: 1064-1067) by Drs. W. B. Kouwenhoven, J. R. Jude, and Mr. G. G. Knickerbocker of the Johns Hopkins University School of Medicine at Baltimore. The method, which they developed through extensive experimentation on more than 100 dogs, has been used with notable success on patients with cardiac arrest.

The new technique is easily applied and, unlike direct cardiac massage, it eliminates the necessity for surgical exposure of the heart. To effect massage, the operator places both hands, one above the other, with the heel of the lower hand resting on the lower third of the patient's sternum and presses vertically downward about 60 times per minute. With each pressure stroke the sternum is moved 3 or 4 centimeters toward the spine, thus compressing the heart and forcing out blood. Relaxation at the end of the stroke allows the heart to fill again. Artificial respiration should be given simultaneously.

"When cardiac arrest occurs," the scientists point out, "the circulation must be restored promptly; otherwise anoxia will result in irreversible damage." They found that closed-chest cardiac massage, combined with artificial respiration, maintains a flow of oxygenated blood to the heart and central nervous system until normal heart action returns spontaneously or, in cases of ventricular fibrillation, until a closed-chest defibrillator can be brought to the scene of the emergency. According to the investigators' experience, hearts in fibrillation for longer than 3 minutes usually do not respond to defibrillation procedures. Closed-chest cardiac massage, however, greatly extended this time limit: "Adequate circulation for periods as long as 30 minutes was easily maintained with the dog in ventricular fibrillation. A closed-chest defibrillating shock would result in the immediate return of normal sinus rhythm in such animals," they explain.

In the 10 months preceding their report, Dr. Kouwenhoven and coworkers applied closed-chest cardiac massage to 20 patients who were from 2 months to 80 years of age. In addition to cardiac massage, which varied from less than 1 minute to 65 minutes in duration, 3 patients required defibrillation and 13 required artificial respiration. The scientists report that all 20 patients were successfully resuscitated and, at the time of this report, 14 patients were alive without central nervous system damage; an over-all permanent survival rate of 70 percent.

Although their data was obtained from hospitalized patients, Dr. Kouwenhoven and coworkers conclude, "The real value of the method lies in the fact that it can be used wherever the emergency arises, whether that is in or out of the hospital."

PORTABLE PACEMAKER AIDS PATIENTS WITH COMPLETE HEART BLOCK

Electrical stimulation of the heart with a transistorized portable pacemaker is reported to be an effective method of treating complete heart block in patients. A description of the apparatus and its use in 66 patients is reported in The Journal of the American Medical Association (172: 2006-2010) by Drs. C. W. Lillehei, V. L. Gott, P. C. Hodges, Jr., D. M. Long, and Mr. E. E. Bakken of the University of Minnesota Medical School at Minneapolis.

In complete heart block, disease or operative injury of the heart's conduction tissue blocks nerve impulse conduction from the sinoatrial node (the heart's "pacemaker" located in the right atrium) to the ventricles. The ventricles thus lose their normal rhythm and frequently cease beating entirely.

The artificial pacemaker developed by the Minnesota workers is designed to maintain the heartbeat for days, weeks or even months--until diseased or injured tissue regenerates and the heart's own pacemaker can resume maintenance of normal heart rhythm. The device consists of a transistorized transformer powered by a self-contained long-life battery, and is connected to the heart by one or two thin wire electrodes inserted into the heart muscle.

Electrode(s) may be inserted either through the open chest or, in nonsurgical patients, by piercing the chest wall with a hollow needle, through which an electrode is passed and the needle subsequently withdrawn. The small pacemaker (slightly larger than a package of cigarettes) is completely portable and is worn strapped to the patient's chest.

The NHI grant-aided scientists report that artificial pacemaker stimulation has proved to be the most effective method of managing complete heart block in surgical patients, and that 2/3 to 3/4 of these patients required less than 2 or 3 weeks of continuous stimulation. Most of the remaining surgical patients were "weaned" from the pacemaker by progressively lowering the rate of stimulation and administering isoproterenol hydrochloride, a drug which increases cardiac rhythmicity and rate.

Most of the patients treated in this series had developed heart block subsequent to cardiac surgery. However, the pacemaker has also been used successfully in the treatment of Stokes-Adams syndrome--heart block due to atherosclerosis, infection, or certain drugs--restoring many of its victims to useful activity. The investigators point out that, not only has the threat of sudden death in these patients been removed, but that their physical and emotional rehabilitation has been dramatic. At the time of their report, one patient with Stokes-Adams syndrome had been maintained under continuous stimulation for 15 months.

NEW AORTIC VALVE PROSTHESES REPORTED BY NHI GRANTEES

University of Minnesota surgeons David L. Long, Jr., Laurence P. Sterns, Robert H. De Reimer, Herbert E. Warden, and C. Walton Lillehei, described in Surgical Forum (10: 660-665) two different types of plastic valves which they developed for the replacement of irreparably damaged aortic valves. They report that one type of valve has been used with success in patients.

Both valves - a flap valve and a bicuspid valve - are constructed of silastic (a rubbery silicone plastic) bonded to a polyvinyl sponge (Ivalon) base. They are inserted through a slit in the aorta and secured by sutures at the site of the natural aortic valve.

Both valves have been tried in dogs. They were also tested in human cadaver hearts which were made to "beat" in a mechanical pulse duplicating apparatus.

Flap valves were successfully inserted by the Minnesota surgeons in two patients, with subsequent restoration of valvular function in each case. The investigators report that the first patient was well and active at the time of their report, eleven months after surgery, and that the second patient was well and active following the operation but died two months later of an unrelated cause. Autopsy revealed a properly positioned, functional valve with no abnormal blood clots present.

Function and durability tests of the bicuspid valve in a pulse duplicating machine have been very encouraging but complications that occurred upon installation of the valve in dogs delayed clinical trials. Difficulties encountered included leakage of blood between the valve and aortic wall in some animals before a suitable method was devised for securing the valve. Complete heart block also developed in some animals as a result of damage to heart impulse-conduction tissue by sutures or internal bleeding. The investigators believe that, in the human heart, the larger size of the structures may make it possible to avoid this complication.

Results of the Minnesota studies suggest, conclude the investigators, "that substantial progress has been achieved in the resolution of many of the very significant problems concerned in the use of artificial cardiac valves. Moreover, this same progress, culminating in successful clinical application, has served to point the way for numerous further improvements in choice of materials, design, and surgical technique."

ARTIFICIAL HEART PROTOTYPES DESIGNED AND TESTED BY NHI GRANTEES

The design and preliminary trials of two prosthetic hearts are reported in the American Heart Journal (59: 723-736) by Drs. C. S. Houston, Tetsuzo Akutsu, and W. J. Kolff, at the Cleveland Clinic Foundation and the Frank E. Bunts Educational Institute in Cleveland. Studies of the mechanical hearts in dogs and in a mock circulatory system indicate that progress is being made toward replacing irreparably damaged human hearts with mechanical pumps implanted within the chest. These pumps, however, are not sufficiently developed to warrant their installation in humans.

Each of the new prostheses--a pendulum-type pump and a roller-type pump--contains an electric motor and two elastic pumping chambers, or ventricles, of polyurethane plastic. Polyurethane valves and connecting tubes maintain unidirectional blood flow through the hearts, and plastic coated wires supply current from an externally located battery pack or wall socket to power the hearts.

In the pendulum pump, a motor suspended on pivots (the"pendulum") swings back and forth within a rigid outer housing, alternately compressing each ventricle against the housing. To-and-fro excursions of the motor-pendulum are caused by an eccentrically-mounted, adjustable lever arm which connects the motor shaft with the outer housing.

Tests of the pendulum pump in a mock circulatory system showed that as much as 1800 ml. of blood per minute is ejected from each ventricle and that hemolysis of red blood cells by the pump is not excessive, report the Cleveland scientists.

Encouraged by these tests, they installed a pendulum pump at the anatomical site of the natural heart in a 30-kilogram dog, which was maintained by a heart-lung machine during removal of its own heart. The investigators report that the dog's circulation was sustained for 5 hours by the pump, but that several infusions of norepinephrine were required to maintain blood pressure. The pump had been adjusted to deliver 2000 ml. per minute which, they explain, was inadequate for a dog of this size. The pendulum pump, however, "seemed promising enough to serve as a model for a more durable pump, which is under construction," they report.

The second type of pump designed by the Cleveland group employs a small, motor-driven roller to compress the ventricles against a rigid outer housing. Polyurethane foam inserted between the ventricles and housing, provides a yielding surface which minimizes hemolysis.

In mock circulation tests of this pump, ventricular outputs increased (in the left ventricle, from 200 to 2,200 ml. per minute) as atrial pressures were increased. Thus, the output on both sides are self-regulating; if one side pumps more blood than the other, the increased atrial pressure on the other side leads to an increased output by that side.

In a single animal experiment, the roller pump sustained a dog for 2 hours. Although the pump was not sufficiently streamlined to allow closure of the dog's chest, there was evidence of spontaneous breathing and of excellent blood oxygenation; pressure tracings indicated good functioning of the pump.

NEW KNOWLEDGE AND METHODS

NEW DIURETIC IS EFFECTIVE AGAINST EDEMA OF NORMAL OR TOXEMIC PREGNANCY

Hydrochlorothiazide, a new diuretic drug derived from chlorothiazide, has been found highly effective against the edema of normal or toxemic pregnancy.

In NHI grant-supported studies at the University of California Medical Center, Dr. N. S. Assali used hydrochlorothiazide, as Esidrex (CIBA) and HydroDiuril (Merck Sharp & Dohme), to treat 33 patients with edema accompanying normal pregnancy and 25 edematous patients with toxemia of pregnancy. His findings, reported in Clinical Pharmacology and Therapeutics (I, 48-52), indicate that the diuretic action of the new drug closely resembles that of chlorothiazide, but appears to be effective at one-tenth the dose.

Edema is the accumulation of fluid in the extracellular tissue spaces. Its most common cause in normal pregnancy is too much salt in the diet or salt retention by the kidneys. Edema in toxemia of pregnancy is also associated with salt retention, and may be abetted by the loss of protein in the urine that also characterizes this disorder. Although slight or moderate edema is more unsightly than clinically dangerous, severe edema can lead to fluid accumulation in the abdominal cavity (ascites) or in the chest, where it can seriously interfere with respiration.

Like chlorothiazide, hydrochlorothiazide reduced edema by increasing renal excretion of salt, potassium, and water; but 100-150 mg. seemed to produce effects comparable to those produced by 1000-1500 mg. of chlorothiazide in a previous study. The drug appears to increase salt and water excretion by inhibiting their reabsorption in the kidney tubules, since it produced no significant effects on renal plasma flow or filtration rates.

The drug produced no observed adverse effects, did not noticeably alter serum electrolyte levels in most patients, and produced most marked diuresis in patients whose edema was most severe. As edema diminished, the effects of the drug were reduced. This is a very desirable feature of the drug because it provides a margin of safety to protect against the possibly serious consequences of electrolyte depletion.

DEVELOPMENTS EXTEND USE OF GAS CHROMATOGRAPHY IN LIPID ANALYSIS

Important new contributions by NHI scientists have made possible the use of gas chromatography for the radioassay of fatty acids labeled with radioactive carbon and for the analysis of steroids. These

developments should greatly facilitate research on lipids and lipid metabolism and the possible roles of these factors in atherosclerosis.

Originally developed in England in 1952, gas chromatography has helped solve many problems of lipid analysis formerly posed by analytical methods that were complex, slow, and insufficiently accurate for the assay of small samples. In gas chromatography, a small sample of the lipid mixture to be analyzed is injected into an analytical column, vaporized, and swept through the column on a stream of inert carrier gas (argon or helium).

In transit through the column the lipid vapors are brought into repeated contact with a non-volatile liquid, present as a thin coating either on the inner surface of the column or on small inert particles with which the column is packed. All of the lipids are soluble in this liquid, but the extent varies with their structure and chemical properties. This variation affords the means of separating the lipid components (the more soluble the lipid, the slower its transit and vice versa) and also for identifying each component as it emerges. As it emerges from the column the lipid vapor enters a special detector which senses its passage, measures its concentration, and records these data against time on a strip chart.

The system for the radioassay of carbon-14 labeled fatty acids by gas chromatography was worked out by Drs. Arthur Karmen and Harold R. Tritch, of the Laboratory of Technical Development. The system, described in Nature (186: 150-151), uses a short column containing anthracene crystals coated with silicone oil to trap the fatty-acid vapors emerging from the analytical column.

The radioactive vapor dissolves in this oil, thereby coming in close proximity to the anthracene crystals. The radiation emitted causes small flashes (scintillations) in the crystals. These scintillations are detected by phototubes and counted by an automatic scintillation counter.

The system allows the collection of individual fatty acid esters in separate trapping columns or for continuous monitoring of a single column. In the latter method, a device measures the rate at which scintillations are occurring and plots this against time on a strip chart. As each new component enters the trapping column, this rate increases sharply. From this data can be determined the time that each component entered the column and how much radioactivity it contained.

The use of gas chromatography for the analysis of steroids was made possible by a special analytical column designed by Drs. W.J.A. VandenHeuvel, C. C. Sweeley, and E. C. Horning, of the NHI Laboratory of the Chemistry of Natural Products.

Steroids, the subject of intense scientific study in recent years, are members of a large family of compounds which include cholesterol, many important adrenal hormones, several vitamins, and the male and female sex hormones. Previous attempts had been made to separate and analyze steroids by gas chromatography, but had been at best only partly successful. Unfortunately, with ordinary liquid phases and column temperatures, most steroids were so soluble in the liquid phase that they would be retained too long in the column. As a result, many heat-sensitive steroids would decompose before entering the detector.

The scientists overcame these problems with a column whose inert particles were coated with a very thin film of methyl silicone gum. The column could be used at low temperatures and gave rapid transit times, a combination which solved the steroid heat-sensitivity problem. At the same time it provided high sensitivity--being able to analyze samples weighing as little as one millionth of a gram--and could separate components differing only very slightly in their molecular structure.

In addition to offering a unique combination of speed, sensitivity, and high resolution, the column has proved to be versatile as well. It has been used successfully to separate and analyze a large number of alkaloids, an enormous family of plant substances which has yielded many valuable drugs over the years.

The column and its uses were reported at the Milan Symposium on Drugs Affecting Lipid Metabolism and in The Journal of the American Chemical Society (82: 3481, 3791).

REPORT GAS CHROMATOGRAPHY DETECTOR BASED ON SOUND VELOCITY VARIATIONS

An engineer in the NHI Laboratory of Technical Development has designed detecting equipment utilizing measurement of sound velocity in gases for characterizing the constituents of samples separated by gas chromatography.

For maximum usefulness as an analytical tool in the biologic sciences, the principle of gas chromatography as introduced from England has required the design and incorporation of more sensitive and versatile systems for detecting the constituents of complex samples once the chromatograph had separated them.

Investigators in the NHI Laboratory of Technical Development have attacked this problem on several fronts with notable success, designing and developing detecting systems which permit extension of gas chromatography to many new research areas.

New detecting equipment using variations of measured sound velocity was described by Frank W. Noble at the Thirteenth Annual Conference on Electrical Techniques in Medicine and Biology held in Washington, D. C. The velocity of sound passing through a gas varies according to the physical properties of the gas, such as its molecular weight. With the equipment described, voltage is applied to a sound-transmitting crystal at one end of a tube containing the sample, and recorded by a receiving crystal at the other end. Phase differences induced by particular samples, between the two voltages are sufficiently definitive to imply analytical usefulness for the principle.

The actual value of the sound velocity principle as used in the equipment Mr. Noble described remains to be demonstrated in applied research.

DRUG METABOLISM BY LIVER UNALTERED IN PATIENTS WITH CIRRHOSIS

Preliminary studies at NHI and New York University suggest that normal rates of drug metabolism are not altered by cirrhosis, despite severe loss of functioning liver, wherein are located the special enzyme systems that inactivate drugs. Thus, the presumed sensitivity to certain drugs of patients with liver disease may require some other explanation, if these indications are borne out by more extended studies.

Conversion of certain fat soluble foreign compounds, such as drugs, into inactive derivatives is an important function of the liver, without which the action of many drugs would persist for a dangerously long time. The liver disposes of such compounds by converting them to derivatives which are less fat soluble, thus barring them from reabsorption into the body through the fat-like membrane lining the kidney tubules.

Recently Dr. Bernard B. Brodie and Dr. John Burns of NHI, and Dr. Murray Weiner of New York University Research Service compared the inactivation of various drugs in patients with severe Laennec's cirrhosis and in normal subjects. The drugs studied (phenylbutazone, aminopyrine, antipyrine, salicylic acid, and dicumarol) had previously been shown to be metabolized in the body through the action of liver enzymes.

The biologic half-life of these drugs was not significantly different in the cirrhotics and controls, indicating that the activity of the enzyme systems involved was not appreciably affected by the liver-damaging disease. Special precautions are usually advised in the administration of certain other drugs to patients with liver disease, for it is commonly believed that drug metabolism is generally impaired in the diseased liver. The present studies should be extended to patients with other types of liver disease as well as to other compounds that are metabolized along similar other pathways. If indications of the present findings are borne out by further study, some other explanation should be sought for the presumed sensitivity to certain drugs of patients with impaired liver function.

These findings were published in Medicina Experimentalis (1: 290-292).

REFLEXES FROM CAROTID SINUS IMPORTANT IN THE CONTROL OF HEART OUTPUT

Reflexes originating in the carotid sinus have been shown by NHI scientists to control the vigor with which the heart's receiving chambers (atria) pump blood into the ventricles. This, in turn, importantly affects ventricular performance and cardiac output.

The carotid sinus has long been known to be sensitive to changes in blood pressure and to initiate nervous reflexes to compensate those changes. However, it was formerly thought that this was accomplished primarily by bringing about changes in peripheral resistance to blood flow and by reflexly changing heart rate. Now studies at the NHI Laboratory of Cardiovascular Physiology have revealed that when arterial pressure falls, the carotid sinus, in addition to reflexly increasing peripheral arteriolar and venous tone and heart rate, also increases the force of atrial contraction.

This is done by diminishing the activity of the vagus nerve (which normally restrains the atrial pulse) while simultaneously increasing the activity of the cardiac sympathetic nerves. Sympathetic stimulation increases the vigor of atrial contraction, presumably by increasing heart levels of catechol amines. These increase the excitability of the atrial muscle fibers and also the synchronization with which they contract.

The increased vigor of atrial contraction pumps more blood into the ventricle, distending it and elongating its muscle fibers more than usual. Since muscle fibers contract more forcefully from longer fiber lengths than from shorter ones (provided that the chemical environment of the fibers is not altered), the result is a more vigorous contraction of the ventricle and hence increased heart output.

The opposite takes place when arterial pressure is high. The carotid sinus reflexly increases vagus activity while decreasing sympathetic activity. This suppresses the atrial pulse, reducing ventricular filling pressure and decreasing heart output.

These reflexes, called the carotido-sympatho-atrial and carotido-vago-atrial reflexes, are held to be of substantial importance in the regulation of the circulation in changing states. They are particularly important at high heart rates, which give the ventricles less time to relax and the atria less time to fill them adequately between pumping strokes. By causing the atria to deliver more blood to the ventricles more rapidly under these conditions, these reflexes help to maintain the heart output at appropriate levels at high-heart rates.

The work leading to the discovery of the carotido-atrial reflexes was done by Drs. Stanley J. Sarnoff, J. P. Gilmore, and J. H. Mitchell. Their findings were reported at the Federation Meetings in Chicago.

ALCOHOL CAUSES DEPOSITION OF FAT IN LIVER BY ACTION ON PITUITARY

NHI scientists have found that large single doses of alcohol in rats interfere with pituitary control over the fat transport system. As a result, excessive amounts of triglycerides (neutral fat) are mobilized from adipose tissue as free fatty acids and carried by the plasma to the liver, where they are recombined with glycerol and deposited in this organ. This derangement of fat transport may be important in the development of the cirrhosis often found in alcoholics, in which the chronic deposition of excessive fat in the liver is thought to lead eventually to necrosis.

Single orally administered doses of 4.8 grams of alcohol per kilogram of body weight (equivalent in humans to about six double martinis) resulted 18 hours later in a threefold increase in liver triglycerides in female rats. Larger doses increased liver triglycerides almost fivefold.

That these effects were due to the mobilization of fatty acids from adipose tissue rather than to an increased synthesis of fatty acids by the liver was shown by the linoleic acid content of the deposited fat. This unsaturated fatty acid cannot be synthesized by the rat, but is of dietary origin. When assayed by gas chromatography, the liver fat deposited by action of the alcohol was found to have virtually the same linoleic content as adipose tissue. Similar results were obtained when the oleic acid content was assayed. Since the

concentration of both fatty acids in liver reflects that in adipose tissue, little if any of the fat deposited as a result of the alcohol could have come from fatty acids synthesized by the liver.

The finding that alcohol did not produce these effects in rats whose pituitaries had been removed suggested that the effects of alcohol on fat transport were mediated through hormones from this master gland. Investigation of the effects of alcohol on the pituitary-adrenal axis indicated that alcohol causes the pituitary to release ACTH and perhaps other hormones important in fat mobilization. However, the mechanism by which alcohol does so is not known.

The scientists found that pretreating rats with certain adrenergic blocking agents prevented the alcohol-induced fat deposition in liver, suggesting that the catechol amines may also be involved in the process. It is not yet clear whether the protection conferred by these agents is due to their blocking the action of amines at nerve endings which innervate fat cells or to their blocking the release of pituitary hormones. Studies of the mechanism or mechanisms involved are underway in the hope that these might lead to drugs that can prevent or ameliorate Laennec's cirrhosis.

The experiments were conducted by Drs. B. B. Brodie, H. M. Maling, W. M. Butler, Jr. and R. P. Maickel, of the Laboratory of Chemical Pharmacology, and by Dr. M. G. Horning, of the Laboratory of Cellular Physiology and Metabolism. Their findings were reported at the Symposium on Neurological and Hepatic Complications of Alcoholism held in New York.

MYOCARDIAL EDEMA RESULT OF HIGH ATRIAL PRESSURE IN CONGESTIVE FAILURE

Studies on the mechanism of myocardial edema--the accumulation of water and electrolytes in the heart muscle that often accompanies congestive heart failure--indicate that the major pathogenic factor is the elevated right atrial pressure also frequently found in this condition. Excessive serum levels of aldosterone, a hormone which promotes water and salt retention, and low serum protein levels may also play a role in the process.

The studies were performed on 46 dogs--21 normals and 25 with experimentally produced lesions causing chronic congestive heart failure or chronic ascites (accumulation of fluid in the abdominal cavity). After periods ranging from 6 to 189 days, the animals were anesthetized, sacrificed, and samples of heart tissue immediately taken for determination of water, fat and electrolyte content.

The experimental data were then analyzed to evaluate the importance of the following factors in myocardial edema: the duration of heart failure, enlargement of the ventricles, serum aldosterone and protein levels, and elevation of right atrial pressure.

All of the experimental lesions produced moderate to marked increases in heart muscle content of water, sodium, and chloride. The observed increases in myocardial chloride suggested that the edema was the result of accumulation of extracellular fluid.

Comparison of data from dogs dying from heart failure within 6 days with data from another group surviving for 3-6 months ruled out duration of heart failure and ventricular enlargement as significant factors in myocardial edema. Other data suggested that high serum aldosterone and low serum protein levels, while not determining factors in themselves, do play supporting roles in the process.

The factor that appeared to be most important in myocardial edema was elevation of right atrial pressure. The degree of increase correlated well with the severity of the edema. Increased right atrial pressure appears to result in passive venous congestion, which, when combined with high serum aldosterone and low serum protein levels, leads to the accumulation of water, sodium, and chloride in heart muscle that constitutes myocardial edema.

These experiments were performed by Drs. N. A. Yankopoulos, J. O. Davis, Ernest Cotlove, and Mary Trapasso of the Laboratory of Kidney and Electrolyte Metabolism. Their findings were reported in the American Journal of Physiology (199: 603-608).

NEUTRAL FAT MADE IN ADIPOSE TISSUE BY MECHANISM SIMILAR TO THAT IN LIVER

NHI scientists have found that fatty acids are converted to their neutral storage form (triglycerides) in adipose tissue by a mechanism very similar to that reported by others for triglyceride synthesis in liver. The studies also indicate that the release of free fatty acids from adipose tissue may be importantly influenced by factors affecting the rate of triglyceride synthesis.

In these studies Drs. Daniel Steinberg, Martha Vaughan, and Simeon Margolis, of the Laboratory of Cellular Physiology and Metabolism, and Dr. Arthur Karmen, of the Laboratory of Technical Development, measured the conversion of a radioactive fatty acid (palmitic acid) to triglycerides and other neutral fat in rat adipose tissue. This tissue was ground up and the homogenized extract divided into two portions. One portion was then

fortified with substances known to favor triglyceride formation; the other portion, unfortified, was used as the control tissue.

The incorporation of radioactive palmitic acid into neutral fat was observed only in the fortified tissue and was almost exclusively in the form of diglycerides and triglycerides. Knowing what substances had been added to the fortified tissue, the scientists were able to determine the probable sequence of biochemical steps by which triglycerides are formed in adipose tissue. This was found to be very similar to the sequence previously reported by others for triglyceride synthesis in liver.

Triglycerides, the form in which most fats are stored in the body, are neutral esters resulting from the combination of three fatty acid molecules with one molecule of glycerol. However, under the biochemical conditions of the body, this combination cannot occur directly. Instead, the fatty acids must first be combined with coenzyme A, which serves as a "carrier" in the process. Two of the combined molecules (fatty acyl-CoA) then react with glycerol, forming a diglyceride and regenerating coenzyme A. With coenzyme A again the "carrier", the third fatty acid is added to the diglyceride to form the triglyceride. The triglyceride thus formed can in turn be broken down to yield its fatty acids to meet the body's energy requirements during the fasting state.

The scientists observed that the release of free fatty acids (FFA) from adipose tissue appeared to be influenced by the rate of triglyceride synthesis. In the fortified tissue, where biochemical conditions favored triglyceride synthesis, there was a net uptake of FFA; however, in the unfortified tissue there was a net release of FFA. A number of hormones are known to stimulate FFA release, and these observations suggested that their actions might be due in part at least to their effects on triglyceride synthesis.

In experiments following up this lead, Drs. Steinberg, Vaughan, and Margolis determined the effects of glucose, ACTH, epinephrine, and serotonin on triglyceride synthesis in intact adipose tissue incubated in a medium containing radioactive palmitic acid.

When glucose, an inhibitor of FFA release, was added to the incubation medium, the incorporation of the radioactive palmitic acid into triglycerides was more than doubled in the tissue sample. However, the addition of ACTH or epinephrine, hormones which stimulate FFA release, reduced triglyceride synthesis by 50% or more. The reduction in triglyceride synthesis was accompanied by increased FFA concentrations both in the tissue samples and in their incubation media. Serotonin, a hormone which does not affect FFA release, also did not affect the rate of triglyceride synthesis. These data thus lend

support to the hypothesis that hormonal control over FFA release is exerted, at least in part, over the rate of triglyceride synthesis.

These findings were reported in Federation Proceedings (19;227) and in the Journal of Biological Chemistry (235; 38-39).

TRANQUILIZER TRIGGERS PITUITARY-ADRENAL "STRESS" RESPONSES

Reserpine, a drug in wide clinical use as a hypotensive and tranquilizing agent, has been found to trigger certain body responses usually associated with stress: hypersecretion of ACTH, increased serum levels of glucocorticoids from the adrenal cortex, and excessive mobilization of free fatty acids from adipose tissue. The paradox implicit in these actions of reserpine is heightened by experimental evidence that these "stress responses" 1) are produced only by doses of reserpine sufficiently large to produce overt sedation, and 2) are the indirect result of the same action of the drug believed responsible for its tranquilizing effects--depletion of brain serotonin stores.

These findings were made by Drs. Bernard B. Brodie, Erik O. Westermann, and Roger P. Maickel, of the NHI Laboratory of Chemical Pharmacology, as part of a series of animal studies on the role of the hypothalmo-pituitary-adrenocortical system in biochemical adaptation. They were presented by Dr. Brodie at the Fourth International Neurochemical Symposium, held at Varenna, Italy.

The scientists found that doses of reserpine which depleted brain stores of norepinephrine and serotonin, producing marked sedation, also provoked a sustained activation of the pituitary-adrenal system. This activation was manifested in rats by: 1) a fall in adrenal ascorbic acid, accompanied by a rise in plasma corticosterone; 2) increased activity of tryptophan peroxidase (TPO), a liver enzyme whose activity is an indicator of pituitary-adrenal stimulation; and 3) increased mobilization of free fatty acids from adipose tissue.

These responses are similar to those produced by the stress of prolonged exposure to cold, and were not produced by reserpine or cold in animals whose pituitaries or adrenals had been removed. This provided further evidence that these responses to reserpine result from pituitary-adrenal activation and are mediated by ACTH hypersecretion.

Hypersecretion of ACTH does not result from the action of reserpine *per se*, but is related to its depletion of brain serotonin stores. Doses of reserpine that deplete brain serotonin

by less than 50% do not elicit noticeable sedation nor hypersecretion of ACTH; slightly larger doses elicit both responses, which become more marked with increasing doses until a maximum is reached with the complete depletion of brain serotonin stores. That ACTH hypersecretion is related specifically to serotonin depletion has been shown by experiments using alpha-methyl-m-tyrosine. Doses of this amino acid that depleted brain norepinephrine stores with very little effect on brain serotonin elicited neither sedation nor ACTH hypersecretion.

This indirect action of reserpine also explains why the "stress" responses, like the drug's sedative effects, persist long after the administered reserpine has disappeared. Apparently, reserpine is a "hit-and-run" drug which depletes the brain of amines by inactivating or destroying their storage sites. The brain amines remain depleted until the storage sites recover, when brain amines slowly return to normal. The "stress" responses will persist until brain serotonin again reaches 50% of normal.

These studies point out the potential value of pharmacological tools in clarifying how the brain directs biochemical mechanisms, and that the interaction of drugs with these mechanisms may be an important aspect of drug action.

RHEUMATIC FEVER STUDIES REVEAL PREDICTABILITY OF HEART DAMAGE

NHI grant-aided studies of rheumatic fever recurrences at a rheumatic fever prophylaxis clinic indicate that: 1) signs of heart valve damage in susceptible patients usually appear during the initial attack of rheumatic fever; and 2) patients free from valvular involvement during the first attack usually remain free from rheumatic heart disease despite recurrent attacks.

These findings were reported in The New England Journal of Medicine (262: 533-540) by Drs. Alvan R. Feinstein and Mario Spagnuolo of Irvington House, Irvington-on-Hudson, New York, and the New York University College of Medicine in New York City.

Their results are based on analyses of 370 documented attacks of rheumatic fever in 161 patients at Irvington House. Only those patients whose attacks fulfilled the modified Jones criteria for the diagnosis of rheumatic fever were included in the study.

The New York scientists found that valvulitis, as determined by careful stethoscopic examination, was a feature of the initial attack in 90 of these 161 patients. With subsequent

attacks, the valvulitis remained unchanged or became worse and in some cases was accompanied by cardiac enlargement, pericarditis, or congestive heart failure.

Valvular involvement was not found in the initial rheumatic episodes of 71 patients. With subsequent attacks, all but 10 of these patients remained free of valvulitis and other symptoms of heart disease. Although 10 patients were found to have a significant diastolic murmur at a subsequent attack, the investigators speculate that in at least 8 of these 10 patients, the murmur was present but not recognized at the first attack.

"The results suggest that the rheumatic host has a specific and persistent susceptibility to get or to avoid valvular involvement. If it does not occur with the first attack, it does not occur (with dubious exceptions) in the subsequent attacks. In most cases in which rheumatic heart disease develops, the valvular involvement is manifested in the very first attack," conclude Drs. Feinstein and Spagnuolo.

Their findings, which should be of considerable interest to the parents of rheumatic children, may enable a reduction in the duration of rheumatic fever prophylaxis for valvulitis-free patients. However, "Prophylaxis should not be stopped in this group without careful auscultatory confirmation of cardiac status and until further studies confirm the present conclusions," state the scientists.

LIPIDS PLAY MAJOR ROLE IN PROTEIN SYNTHESIS, NHI STUDIES SUGGEST

Evidence for a major role of intracellular lipids in protein synthesis has been gained from studies by Dr. Richard Hendler of the NHI Laboratory of Cellular Physiology and Metabolism. He reported these findings at the Federation Meetings.

In the search for the intermediate through which the stored genetic master plans for each protein molds and binds its amino acid structural units into the finished molecule, protein chemists in many countries are concentrating on variants of the nucleic acids, DNA and RNA. Genetic DNA, in the nucleus of the cell, is widely regarded as the storehouse for these master plans. RNA, located out in the microsomal structures of the cell, is widely regarded as a template, or "die" in which the protein is cast.

Dr. Hendler turned instead to the lipids, which he notes, are even more abundant than RNA in some of the microsomal structures thought to be sites of protein synthesis. (The ribosomes,

richest of all in RNA, have been reported to contain about five times as much lipid as RNA).

From studies of synthesis of the egg protein, albumin, by the hen oviduct, Dr. Hendler has gained evidence that the basic amino acid structural units of proteins combine with lipids very early in the protein assembly process to form more complex units (amino acid complexes). So far eleven albumin amino acids have repeatedly been seen to combine with the lipids in these complexes. The findings suggest the possibility that the lipid-associated amino acid groups may actually represent primitive precursors of the natural protein molecule.

From the evidence accumulated, Dr. Hendler speculates that the basic building blocks of the protein (the amino acids), arriving at the cell via the watery fluids of the body, are made fat soluble, entering into the lipid mechanism very early in the protein assembly process. It is in this lipid medium that their first orderly grouping seems to occur--perhaps the first step in the precise forging of the complex links (polypeptides) that will make up the great chains of the finished protein.

Dr. Hendler is also considering the possibility that the whole assembly process--genetic "master plan" to emergence of the finished molecule--is a lipid process, the study of which might fill major gaps that remain in current understanding of how proteins are made. With this as a working hypothesis, he is continuing in his attempts to sift out recognizable protein precursors in new lipid-associated amino acid complexes.

He is also hoping to find the chemical process by which the individual amino acids are made fat soluble, for this might be the key step which introduces the raw materials of the protein--the amino acids--into the cell's machinery for making the protein.

MYOSIN MOLECULE CONSISTS OF THREE TIGHTLY COILED PROTEIN CHAINS

Recent advances in protein chemistry at NHI and elsewhere permit the conclusion that the myosin molecule is made up of three identical chains, each wound in a tight coil and all three twisted together as a long rope.

The protein myosin is of interest in terms of cardiovascular functions because it is believed to be the predominant component of the contractile system of muscle. A program of

research on myosin at NHI has the immediate objective of interpreting some of the properties of myosin on the basis of its molecular structure, and is ultimately aimed at understanding its role in the contraction of muscle.

Like other proteins the myosin molecule is made of amino acid groups, bound together as links in the long chains which constitute its primary structure. Although myosin is known to be a comparatively long slender molecule (1600 x 22 Angstrom units), it has not been known how many polypeptide chains are held together by secondary bonds to form the molecule and, if more than one chain, whether or not they differ from one another.

Drs. W. Wayne Kielley and William F. Harrington, in the NHI Laboratory of Cellular Physiology, have approached this problem using guanidine hydrochloride to break the secondary bonds holding the chains together, while leaving intact the primary bonds holding together the polypeptide links of each chain.

Examining the products of this dissociating process by sedmentation in the ultracentrifuge, together with observations of their diffusion rates, viscosities, optical rotation properties, and other data, the investigators have concluded that the myosin molecule consists of three identical polypeptide chains, each wound into a tight helical coil, the three chains themselves being wound together in the form of a long rope.

These findings were published in Biochimica et Biophysica Acta (41: 401-421).

NATURE OF HORMONE CONTROL OVER FATTY ACID RELEASE EXPLORED BY NHI STUDIES

Recent studies at the National Heart Institute have explored the mechanisms whereby fatty acids are stored in the body's fat depots as triglycerides (neutral fat) or are released from these depots as unesterified fatty acids (UFA) to meet the body's energy requirements. The studies sought to clarify the mechanism or mechanisms by which four hormones--epinephrine, norepinephrine, ACTH, and glucagon--stimulate UFA release from adipose tissue.

Triglycerides, the form in which most fats are stored in the body, are neutral esters formed by the combination of three molecules of fatty acid with one molecule of glycerol. The

triglyceride can in turn be broken down to yield three molecules of UFA, an important metabolic fuel during the fasting state. The latter process is known to be subject to a number of hormonal controls.

Seeking to clarity the nature of these hormonal controls, Dr. Martha Vaughan, of the Laboratory of Cellular Physiology and Metabolism, studied the effects on adipose tissue of four hormones shown by others to stimulate the release of UFA: epinephrine, norepinephrine, ACTH, and glucagon. Using rat adipose tissue incubated in a medium containing albumin and glucose, Dr. Vaughan found that hormonal stimulation of UFA release was accompanied by increased uptake of glucose by the tissue and by stepped up phosphorylase activity.

Phosphorylase is an enzyme which breaks down glycogen, the form in which carbohydrates are stored in the body, to yield glucose, an important energy source. In the liver, the resulting glucose can be released into the blood by action of the enzyme phosphatase. However, the phosphatase is not present in adipose tissue, so that the small amounts of glucose resulting from phosphorylase activity must be used locally, either as a source of energy or for conversion into fatty acids and triglycerides.

The fact that all four hormones stepped up phosphorylase activity at the same time that they stimulated UFA release suggested that the hormones might control UFA release by a common mechanism. Phosphorylase is not the common denominator, however, since previous work at this laboratory had shown that serotonin, a hormone which increased phosphorylase activity, did not stimulate UFA release.

But the increased phosphorylase activity produced by ACTH in adrenal tissue and by epinephrine and glucagon in liver does have a common denominator--cyclic 3,5 adenylic monophosphoric acid. The hormones have been shown by others to stimulate the accumulation of this compound, which in turn increases active phosphorylase. It is probable that the compound may play a similar role in adipose tissue in the intact animal, even though the compound produced no demonstrable effects on phosphorylase in the <u>in vitro</u> studies.

It is also possible that 3,5 AMP may play a role in hormone-stimulated UFA release, perhaps by affecting reactions in addition to that producing increased phosphorylase.

These findings were reported at the meeting of the American Society of Biological Chemists.

ACTIVE ION TRANSPORT IN RED CELL GHOST POWERED BY ATP

Studies by Dr. Joseph Hoffman, of the NHI Laboratory of Kidney and Electrolyte Metabolism, have shown that Adenosine triphosphate, a compound that powers many of the body's energy-consuming processes, is also the direct energy source for active ion transport in the red blood cell. His findings were reported at the Federation Meetings.

Active ion transport is the process whereby the cell can actively and selectively transport electrically charged substances (ions) across the cell membrane against opposing electrochemical forces. In this process, the "ion pump" maintains normal electrolyte balance within the cell by using ATP generated by cell metabolism to offset the loss of ions across the cell membrane by passive diffusion.

Presumably because one ion cannot replace the metabolic functions of another, the cell must maintain the proper concentrations of the proper ions both for the regulation of its volume and for its metabolic operations. This would be impossible but for the delicate balance struck between passive loss on the one hand and active transport on the other.

Previous work on intact red blood cells by Dr. E. T. Dunham, formerly of the same laboratory, had indicated that ATP might be the fuel for the ion pump. In studies following this lead, Dr. Hoffman used red cell "ghosts" whose normal contents had been considerably reduced by hemolysis. The ghosts not only retained the membrane characteristics of the intact cell, but could also be reconstituted containing known substances whose metabolism could subsequently be observed without the interference that might result from other metabolites and metabolic processes in the intact cell.

Dr. Hoffman's studies showed that ATP was the specific fuel for the ion pump in the ghost system; however, if no ATP was incorporated into the ghost, the pump would still work (though less efficiently) if other substances that would generate ATP were incorporated. These findings suggest that the ion pump requires the presence of an enzyme (an ATPase) in order to use ATP. Such an enzyme has been characterized in membranes isolated from red blood cells by Dr. R. L. Post of Vanderbilt, and appears to possess the same activation characteristics as does active ion transport in both red cells and other ghosts. Thus the evidence is strong that the ion pump either <u>is</u> an ATPase, or else has an ATPase as an intimate component.

PROTEIN AND FAT FRACTIONS OF LIPOPROTEIN MOLECULE PROVIDED BY LIVER

Scientists at NHI have found that the liver, previously shown to be the source of the lipid portion of lipoproteins, is also the source of the protein of this complex molecule, the form in which fats are transported in the blood. Experiments conducted by Drs. Charles Radding and Daniel Steinberg, of the Laboratory of Cellular Physiology and Metabolism, have demonstrated that rat liver slices, incubated in vitro, synthesize and secrete high-density lipoproteins identical with those found in normal rat serum. Their findings are reported in The Journal of Clinical Investigation.

The liver slices were incubated in rat serum with a complete mixture of amino acids labeled with carbon-14. After incubation periods ranging up to four hours, the slices were removed and samples of the media ultracentrifuged to separate the lipoprotein fractions. These were then analyzed by the "fingerprint" technique.

When broken down by the proteolytic enzymes trypsin or chymotrypsin, each protein yields its own characteristic peptide degradation products. These can be spotted on filter paper and separated on the vertical axis by descending paper chromatography, then on the horizontal axis by electrophoresis to form a characteristic peptide pattern. When this pattern is developed by ninhydrin staining, the result is a distinctive "fingerprint" which identifies the protein.

The ninhydrin fingerprints of high-density lipoproteins from the incubation medium were compared with fingerprints of lipoproteins of the same density class from normal rat serum and found to be identical. Autoradiograms (made by placing the media fingerprint strips in contact with X-ray film for 1-2 months, then developing the film) showed the presence of radioactivity in all of the ninhydrin spots of the fingerprint.

These comparisons showed that the lipoproteins synthesized by the liver slices were identical to those of normal serum; and, since labeled amino acids had been incorporated into all of the newly synthesized lipoproteins, that the liver was the source of the protein as well as the lipid portions of those lipoproteins.

To determine whether the rate of lipoprotein synthesis is affected by the rate of cholesterol synthesis, the scientists pretreated rats to speed up or slow down their cholesterol production. Liver slices from these rats were then incubated

with acetate (a cholesterol precursor) and an amino acid, both tagged with radioactivity so that the rates of cholesterol and lipoprotein synthesis could be measured simultaneously.

Lipoprotein synthesis was not noticeably affected even by wide variations in the rate of cholesterol synthesis in these experiments, which suggested that the two rates were independent of one another. This was not proved conclusively, however, because of the possibility that enough cholesterol might already have been formed in the tissue before incubation was begun to support normal rates of lipoprotein synthesis during the short period of experimental observation, but not over extended periods.

Other experiments showed that liver slices from nephrotic rats synthesized both lipoproteins and other serum proteins more rapidly than did those from normal rats. This suggests that overproduction of lipoproteins may be an important factor in the hyperlipemia of nephrosis.

OBSERVE ACCELERATED DRUG METABOLISM IN PRETREATED RATS

Pretreating rats with a variety of drugs has been found by NHI scientists to increase the activity of drug-metabolizing enzyme systems in the liver, thus decreasing the duration of drug action in the body.

The findings were reported in the Federation Proceedings (19: 390) by Drs. A. H. Conney, I. A. Michaelson, and J. J. Burns of the NHI's Laboratory of Chemical Pharmacology. This Laboratory is conducting a broad program of basic studies aimed at elucidating the factors regulating duration of drug action. Previous studies in this laboratory had shown that special enzyme systems in liver microsomes, tiny particles in liver cells, inactivate many drugs as well as other compounds foreign to the body by converting them into compounds which the kidney can excrete.

In the currently reported studies, Dr. Conney and co-workers investigated the ability of microsomal enzyme systems in rat livers to metabolize several commonly used drugs following repeated doses of the same or another drug. Rats were pretreated for four days and their livers incubated with test drugs to determine microsomal enzyme activity. The scientists

also tested enzyme activity in live, pretreated rats by measuring the duration of action of test drugs. Drugs used in the study included barbiturates, muscle relaxing drugs, analgesics, antirheumatic agents, a carcinogenic hydrocarbon, and an antihistamine.

Results of the liver incubation studies showed that pretreatment with phenobarbital, barbital, phenylbutazone, orphanedrine, or aminopyrine caused "several-fold increases" in the ability of liver microsomes to metabolize hexobarbital, zoxazolamine, phenylbutazone, and aminopyrine, report the investigators. They obtained evidence of accelerated drug metabolism in living, pretreated rats by demonstrating a shortened duration of action of hexobarbital, zoxazolamine, meprobamate, and carisoprodol in animals that had received repeated doses of these drugs.

In other in vitro studies, the investigators observed that pretreatment with the hydrocarbon, 3,4-benzpyrene, stimulated the microsomal enzyme system that metabolizes zoxazolamine, but not the hexobarbital-metabolizing system. "In accord with this, 3,4-benzyprene administration shortens the duration of zoxazolamine paralysis from 730 minutes to 17 minutes but does not shorten the duration of hexobarbital hypnosis," they report. Interestingly, the duration of action of both zoxazolamine and hexobarbital was shortened in rats pretreated with the antihistaminic drug, chlorcyclizine.

"The findings that pretreatment of animals with one drug can speed up the metabolism of the same or another drug may be of considerable importance when evaluating the pharmacological activity of drugs that are given repeatedly, either alone or in combination," stated the Heart Institute scientists.

PREDNISONE-ASPIRIN EQUALLY EFFECTIVE IN PREVENTING RHEUMATIC HEART DAMAGE

Cooperative clinical studies have shown that large doses of the steroid, prednisone, are not superior to therapeutic doses of acetylsalicylic acid (aspirin) in preventing residual rheumatic heart disease. The NHI grant-aided studies were conducted by the Combined Rheumatic Fever Study Group which consists of 12 investigators in Children's Cardiac Services of 8 hospitals: 4 in New York City, 2 in Baltimore, and 1 each in Boston and Cleveland. The investigators, coordinated by Dr. A. G. Kuttner ot New York University - Bellevue Medical Center, report their combined findings in the New England Journal of Medicine (262: 895-902, 1960).

Their findings are similar to those of an earlier cooperative study of aspirin vs. steroid therapy, sponsored jointly by the Research Council of Great Britain and the American Heart Association. In the present study, however, steroids were given in larger doses and over a longer period of time.

Admitted to the study were 57 patients who met the following criteria: 1) they were 12 years of age or younger, 2) had suffered their first attack of rheumatic fever not more than 28 days previously, and 3) had moderate to severe carditis as evidenced by pericardial rub or effusion, cardiac enlargement, congestive heart failure, and/or significant heart murmurs. About half of these patients received large daily doses of prednisone which totalled 3 grams over a 12-week treatment period; the remaining patients were given doses of acetylsalicylic acid sufficient to maintain blood levels at 25 to 35 mg./100 cc., also for a 12-week period. Three weeks after termination of therapy and again 1 year later all patients were re-examined and the incidence of residual heart disease in each group compared.

The study group found that both prednisone and aspirin controlled acute rheumatic symptoms in most patients, and, during the ensuing year, all patients remained free of rheumatic recurrences. Of those patients available for study at the end of the year, 12 in the prednisone group and 16 in the aspirin group recovered completely with no signs of residual rheumatic heart disease, whereas 16 prednisone and 7 aspirin-treated patients had residual heart disease at this time. Of 3 patients originally allocated to the aspirin group but later switched to prednisone, 1 recovered completely and 2 were left with residual heart disease.

Thus, "large doses of prenisone given for 12 weeks were not found to be superior to acetylsalicylic acid in preventing residual rheumatic heart disease," conclude the investigators. "The chief action of both prednisone and salicylates appears to be to suppress the inflammatory reaction caused by this disease. In many patients, however, even if the acute symptoms are promptly and well controlled, cardiac damage is not prevented," they state.

In view of these findings, all but one of the investigators felt the risk of prolonged steroid therapy was unwarranted, although no serious side effects of prednisone were noted in this study.

PROGRAM HIGHLIGHTS

WEEK-LONG GRADUATE COURSE IN CARDIOVASCULAR DISEASE GIVEN AT HEART INSTITUTE

"Current Research in Cardiovascular Disease," a one-week postgraduate course sponsored by the American College of physicians, was given at the National Heart Institute in May. 150 physicians attending the course came from a widespread area, with almost every State in the Union being represented, as well as Canada and Puerto Rico. Nine physicians from the PHS Division of Hospitals attended as guests of the Heart Institute and as part of its cardiovascular training program.

Dr. Luther L. Terry, Assistant Director, NHI, directed the course, and 42 Heart Institute staff members participated.

Focused around the research program of the Heart Institute, the course was aimed at acquainting internists with some of the many facets of cardiovascular disease currently under investigation. The faculty discussed the research most applicable to clinical medicine, and supplemented this material with background information and reports on related work in other institutions.

FLU VACCINE RECOMMENDED FOR HEART PATIENTS BY NHI AND AHA

In October the National Heart Institute and the American Heart Association publicized the need for routine vaccination against influenza for persons with heart and blood vessel disease. The action was taken in support of a PHS program to intensify efforts to encourage vaccination of selected population groups where hazards from influenzal infections are particularly great.

A joint statement released by Drs. A. Carlton Ernstene, President, AHA, and James Watt, Director, NHI, reads:

"The epidemics of influenza which occurred in the fall of 1957, the spring of 1958, and the first quarter of 1960 have again emphasized the fact that individuals with cardiovascular or pulmonary disease are more susceptible to the hazards of influenza than is the general population. The increased risk is shown both by more severe illness and by higher fatality rates among patients with these diseases.

"Evaluation studies with influenza virus vaccine have shown that its use is of definite value in preventing influenza.

In adults, side reactions have been extremely few and use of the vaccine is contraindicated only in those patients who are allergic to components of the vaccine. The Public Health Service Advisory Committee on Influenza Research has strongly recommended that those persons at high risk of death from influenza obtain immunization as a protective measure. Because influenza epidemics recur in unpredictable cycles, and particularly because some influenza occurs continually, annual immunization has been recommended.

"The American Heart Association and the National Heart Institute wish, therefore, to bring these facts to the attention of all physicians. In addition, it is urged that persons with cardiovascular disease consult their physicians as to the advisability of obtaining routine influenzal vaccination."

PRIMATE STATION UNDERWAY AT UNIVERSITY OF OREGON; STRONG INTEREST NOTED

Construction began during 1960 of the first regional subhuman primate research center under the new Primate and Veterinary Grants Program. Located near Portland, Oregon, the center is being built with funds from an NHI grant of $1,792,000. A separate grant of $125,275 was awarded at the same time for initial operation of the center. Principal investigators are Donald E. Pickering, M. D. and Edward S. West, Ph.D., both of the University of Oregon Medical School faculty.

Other primate research centers are planned, at locations to be selected in the near future. Since the beginning of the program in 1959, following a special congressional allotment of Heart Institute funds for construction and initial operation of the centers, a number of grant applications have been received from interested scientific groups at various universities.

The new centers are intended to provide ideal environments where both resident and visiting scientists, representing a breadth of disciplines, may pursue research that will advance all areas of knowledge relating to the biological characteristics of the primate throughout its entire life span. The centers will offer opportunities for fundamental research as well as studies more directly related to a variety of human diseases.

SCIENTIFIC EXCHANGE PROGRAM BEGUN WITH SOVIETS BY HEART INSTITUTE

Four Soviet physicians visited NHI in May to participate in planning a joint program of scientific cooperation in the field of heart disease. Their two-week visit included talks with officials and consultants of the Heart Institute, and a tour of scientific institutions in Bethesda, Baltimore, Boston, Cleveland and New York.

Following Chairman Krushchev's 1959 visit, the U. S. and the U.S.S.R. signed an agreement (November 21, 1959) for cooperation in the scientific, technical and cultural fields. Under the Public Health and Medical Sciences portion of the agreement, the U.S.S.R. Ministry of Health and the U.S. Public Health Service were directed to facilitate cooperation between research organizations in the two countries on various important problems of medicine. In the area of cardiovascular disease, the two institutions designated were NHI and the Institute of Therapy, Academy of Medical Sciences, Moscow.

The meetings were considered highly fruitful by members of the two groups, and a number of agreements were reached.

The NHI and the Institute of Therapy each agreed to prepare a list of subjects and areas in which they are particularly interested in exchanging workers. A specific person was to be selected by each side to serve as the focus for exchanges in a particular disease area.

Recognizing the importance of nomenclature and classification of cardiovascular diseases, each side agreed to prepare material and suggestions on this subject.

Each side also agreed to proceed with plans for the exchange of individual workers, and to speed and increase the exchange of medical journals, books and films.

The Russian party, headed by Dr. Alexander Myasnikov, Director of the Institute of Therapy, Academy of Medical Sciences, Moscow, included: Dr. Ivan Speranskiy, Vice-Director of the Institute of Therapy, Academy of Medical Sciences, Moscow; Dr. Mariya Bavina, Biochemical Laboratory in the Institute of Therapy, Academy of Medical Sciences, Moscow; and Dr. Nodar Kipshidze, Member of the Academy of Medical Sciences, and Professor and Head of the Faculty of Therapy Clinic, Tbilisi. Dr. Boris Ilinskiy, Therapy Section, Institute of Physiology, Pavlov Academy of Science, Leningrad, was unable to come due to illness.

The United States participants, headed by Dr. James Watt, Director, National Heart Institute, included Dr. Paul D. White, Emeritus Clinical Professor of Medicine, Harvard Medical School, Boston; Dr. E. Cowles Andrus, Associate Professor of Medicine, and Chief, Division of Cardiology, Department of Medicine, Johns Hopkins Hospital, Baltimore; Dr. George E. Burch, Chairman, Department of Medicine, Tulane University School of Medicine, New Orleans, Louisiana; Dr. Michael E. De Bakey, Chairman, Department of Surgery, Baylor University, Houston; Dr. Irvine H. Page, Director of Research, Cleveland Clinic, Cleveland; Dr. David D. Rutstein, Professor and Head, Department of Preventive Medicine, Harvard University Medical School, Boston; Dr. Luther L. Terry, Assistant Director, National Heart Institute, Bethesda; Dr. Robert W. Wilkins, Professor of Medicine, Boston University School of Medicine, Boston; and Dr. Irving S. Wright, Professor of Clinical Medicine, Cornell University Medical School, New York City.

Later in the year, Dr. Luther L. Terry, Dr. Paul D. White, and Dr. E. Cowles Andrus met again with members of the Russian heart delegation, following the Third European Congress of Cardiology in Rome. Discussions were held of the material that had been exchanged dealing with classification and nomenclature of atherosclerosis, ischemic heart disease, and hypertension. Also discussed were future information exchanges and possible cooperative studies.

NHI CONTINUED SUPPORT AND PARTICIPATION IN SCIENTIFIC MEETINGS

NHI contributed substantially in 1960 to world medical and scientific communication through participation in, and grant support of, conferences, symposia and workshop sessions preparatory to conferences. The meetings afforded invaluable opportunities for the interchange of scientific information, as well as for the planning of collaborative studies and new projects.

Seven of the scientific meetings were held outside the United States with the Heart Institute providing travel and maintenance funds for some participants, and contributing toward operating expenses, such as rent for conference rooms, simultaneous translation and public address systems, printing expenses, and recordings of proceedings. NHI aided in making five of the U. S. meetings international in character by providing funds for travel and maintenance of foreign as well as American participants.

Major NHI-aided conferences of the year were: (1) a Conference on Thrombolytic Agents, Chicago, April 8-9; (2) a Symposium on the Pathogenesis of Essential Hypertension, Prague, Czechoslovakia, May 22-29; (3) the Second Asian-Pacific Congress of Cardiology, Melbourne, Australia, May 31-June 3; (4) the Fifth International Congress of Gerontology, San Francisco, Aug. 7-12; (5) the Sixth Interamerican Congress of Cardiology, Rio de Janeiro, Aug. 14-20; (6) the First International Congress of Nephrology, Evian, France and Geneva, Switzerland, Sept. 1-3; (7) two international congresses in Tokyo on hematology, Sept. 4-10, and blood transfusion, Sept. 12-15; (8) a Conference on Prosthetic Valves for Heart Surgery, Sept. 9-10; and (9) a Conference on Thrombolytic Activity and Related Phenomena, Princeton, Sept. 18-21.

Three of the foregoing had NIH study section sponsorship. The Hematology Study Section sponsored the Chicago Conference on Thrombolytic Agents, and was a joint sponsor with the International Committee on Blood Clotting Factors of the Princeton Conference on Thrombolytic Activity and Related Phenomena. The Conference on Prosthetic Valves for Heart Surgery was sponsored by the Surgery Study Section.

Items of Interest on Program Developments and Research Studies Conducted and Supported by the National Institute of Allergy and Infectious Diseases

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VIRUS DISEASES

VACCINE AGAINST MEASLES PROMISES IMMUNITY COMPARABLE TO NATURAL DISEASE

Impressive evidence of effectiveness of an experimental virus vaccine for measles is presented in eight consecutive reports in the New England Journal of Medicine. These articles represent the work of 23 investigators working in 11 institutions which are receiving grant support from 7 organizations.

The investigators agree that any proposed vaccine for measles must be justified as both desirable and acceptable. In measles, because of the significant mortality, frequency of bacterial complications, occasional involvement of the central nervous system, widespread morbidity of the uncomplicated disease, heightened virulence of outbreaks in isolated populations and unfavorable effects on certain other pre-existing illnesses, a preparation conferring immunity comparable to natural measles seems desirable.

It is the consensus that the present experimental vaccine may be acceptable because of the following properties: ease of administration; lack of local reaction; absence of communicability; high index of serologic response; elimination of bacterial complications; and prophylactic efficacy demonstrated under the circumstances described.

The magnitude of the measles problem is indicated by the following statistics. In 1958, the last year for which complete figures are available, 552 deaths in the United States were attributed to measles in contrast to 255 for poliomyelitis. A dreaded complication of measles is

encephalitis, reportedly ranging in incidence from 1:400 to 1:1000 cases. With an attack rate of 70 to 95 percent among persons susceptible to measles and with a U. S. birth rate of 4 million annually, the morbidity of uncomplicated disease alone is immense and the proportion liable to severe complications considerable.

The work on the vaccine supported by the National Institute of Allergy and Infectious Diseases (articles IV. and V. in the series) is concerned with two aspects of the study. Drs. F. L. Black and S. R. Sheridan of Yale University School of Medicine compare the effectiveness of the subcutaneous route of injection with others that might be involved in natural transmission of measles. Drs. M. L. Lepow and F. C. Robbins, with Nigel Gray, report the clinical, antigenic and prophylactic effects in institutionalized and home-dwelling children.

Results obtained in the trial of subcutaneous vaccine, which proved superior to inoculation by other routes, were in keeping with those of other investigators: a predictable incubation period shorter than in natural measles; a roseola-like course of illness without rash and fever; no cough, coryza and conjunctivitis; surprising lack of malaise and prostration when fever was present. Production of antibodies was reliable and leukopenia was a relatively constant sign of response to the vaccine. No contact spread was seen.

In the study by investigators Lepow, Gray and Robbins, as in the others reported, the majority of the systemic reactions were mild, according to the physicians and the parents of the study children. Fever, anorexia and lethargy were predominant features of the 15 percent of reactions considered moderate. Serologic data indicate that an adequate immune response does occur, and thus far complete protection has been demonstrated in 10 children after household exposures to the natural disease. None of the children who had detectable complement-fixing antibody in their preimmunization serum samples showed any rise in titer as a result of administration of the vaccine. Parents who had seen natural measles in other children considered the vaccine-induced reaction in the study group considerably milder.

One problem that complicated assessment of the rate and severity of the reactions to the vaccine was the presence of intercurrent infections suffered by one-fourth of the susceptible children during the 14-day post-vaccination period. It is quite possible, the authors believe, that some of the respiratory symtoms attributed to vaccine resulted from other infectious agents. There was no evidence, however, that intercurrent infections interfered in any way with antibody response to the vaccine.

Parental acceptance of the vaccine will depend on an awareness by parents of the hazards attending ordinary measles as well as preparation for the expected reactions to the vaccine. Laboratory efforts are continuing in an attempt to provide an agent that may induce comparable serologic response with less febrile reaction. At this time the investigators believe it appropriate to recommend extension of clinical trails of attenuated measles-virus vaccine in special groups and a conservative extension of its use in normal children.

28 PERCENT OF RESPIRATORY VIRAL ILLNESS IN YOUNG ADULTS NOW IDENTIFIABLE

Among young adults at the University of Wisconsin, the measure of acute respiratory disease which can be identified etiologically has been raised to over 28 percent by Dr. Alfred S. Evans, a grantee of the National Institute of Allergy and Infectious Diseases. The report, published in the New England Journal of Medicine, evaluates the importance of the hemadsorption viruses in the total picture of respiratory disease.

Dr. Evans' achievement represents another advance in the effort to clarify the etiological problem of undifferentiated acute respiratory disease. In previous work, Dr. Evans had identified about 20 percent of these illnesses etiologically, including streptococcal infection (7.0 percent), influenza infection (8.7 percent), adenovirus infection (1.4 percent), and other bacterial infection (1.2 percent). Results of the present study serve to identify still another segment of the etiologic "pie" in the order of 8 to 10 percent and mark the hemadsorption viruses as the most frequent cause of sporadic respiratory infection found in the student body to date.

The scientist reported on 227 paired serums from students (age 17 to 26) hospitalized in the student infirmary with respiratory illness from January 1957 through April 1959. Cases occurred throughout the year although the periods following summer and Christmas vacations contained a greater portion of the total.

In 200 patients tested by complement-fixation for hemadsorption type 1, 8.5 percent gave evidence of infection. For hemadsorption type 2, the figures were 1.5 percent of 202 patients. In serums from 175 patients on which both tests were made, a total infection rate of 9.1 percent was obtained.

Clinical diagnoses associated with these infections included acute respiratory disease, acute pharyngitis, acute tonsillitis and pneumonitis. However, no distinctive clinical pattern permitting recognition of the cause of infection at the bedside could be detected.

MUMPS VIRUS IN CENTRAL NERVOUS SYSTEM MAY BE WRONGLY DIAGNOSED AS PARALYTIC POLIO

According to Dr. Edwin H. Lennette and cooperating scientists, working under a grant from the National Institute of Allergy and Infectious Diseases, a central nervous system involvement with mumps virus may be incorrectly diagnosed as paralytic poliomyelitis. Results of this investigation are published in Pediatrics.

Although attention has already been focused on the differentiation of disease of the central nervous system due to mumps from nonparalytic poliomyelitis, some cases are observed in which muscle weakness and paralysis occur. These cases are usually diagnosed on the basis of the simultaneous presence of parotitis and muscle weakness, or on positive serologic tests for mumps in a paralytic poliomyelitis-like syndrome. However, in previous studies, the possibility of concurrent poliomyelitis had not been ruled out.

In the current investigation the scientists observed cases of mumps virus infection in children associated with local muscle weakness resembling that of mild paralytic poliomyelitis. In all instances this weakness was still present at the time of hospital discharge; in four cases it was still detectable 2 months after discharge. All of the patients had been initially diagnosed and reported as spinal paralytic poliomyelitis.

Of the four cases displaying a residual weakness after discharge, two gave no laboratory evidence of current or past poliomyelitis virus infection. In the others, failure to recover poliomyelitis virus from the stools, together with the absence of demonstrable CF antibody, was considered strong evidence against recent infection. In each case the diagnosis of mumps infection was based on the occurrence of a four-fold or greater rise in the titer of mumps antibody during the illness.

For the seven remaining cases, a diagnostically significant rise in mumps CF antibody was shown. Again no evidence of current poliomyelitis virus infection, either by isolation of the virus or demonstration of a significant rise in CF or neutralizing antibodies, was found. While low titers of CF antibody and substantial neutralizing antibody titers against one or more types of poliomyelitis virus were present in both acute and convalescent sera from these patients, antibody levels such as these are commonly found in children who have experienced subclinical infection with poliomyelitis virus at some time in the past and have subsequently had their natural antibody "boosted" by vaccine. Thus, although the possibility of concurrent poliomyelitis is not unequivocally excluded in these latter cases, the investigators offer them as additional examples of mild paralytic illness in which the mumps virus is very likely the etiologic agent.

VIRUSES MAY BE IMPORTANT CAUSE OF SHIPPING FEVER IN CATTLE

Parainfluenza 3, a virus closely related to a common human viral agent, has been isolated from cattle with the respiratory disease known as shipping fever, adding weight to the thesis that viruses may be an important cause of this infection.

From 1957 through 1959 in seven separate outbreaks of respiratory disease in calves and older cattle, thirteen isolations of this agent were made from nasal secretions and diseased lungs of the sick animals and from the nasal secretions of three apparently normal calves. The viruses were isolated on tissue culture of bovine embryonic kidney and were identified by the hemadsorption-inhibition technique. Although serological neutralization tests were done, hemagglutination-inhibition was found to be equally sensitive and was therefore used extensively throughout the studies.

A report on this research appears in the American Journal of Hygiene. Co-authors are Drs. F. R. Abinanti and R. J. Huebner, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases; Drs. R. J. Byrne, L. J. Poelma, and F. R. Lucas, University of Maryland Department of Veterinary Science and the Maryland Livestock Sanitary Service at College Park; and Dr. R. L. Watson, Communicable Disease Center, Atlanta.

Prior to recovery of this virus from cattle, a closely related virus, which is apparently common in humans, was shown to be responsible for respiratory illnesses in infants and young children. In a study published last year, parainfluenza 3 virus was shown to have caused 50 percent more acute respiratory disease in hospitalized children than the virus of Asian influenza.

The authors point out that the study of respiratory disease in cattle is of great importance not only to the livestock industry, which must cope with the economic aspects of animal disease and death due to such outbreaks as shipping fever, but to those interested in human viral disease. The investigators believe that respiratory diseases in cattle can serve as useful models of respiratory disease in humans. Rates of infection and the influence of the immunological and physiological state on these rates may be studied, as may the relationship of clinical infection to clinically inapparent infection, seasonal prevalence of disease, and physical factors concerned with the spread of respiratory infections. With a bovine population it is also possible to do detailed studies on the use of vaccines, assessing their usefulness in the control of respiratory disease.

With regard to the isolation of virus from apparently normal calves, the investigators note that their current studies do not establish whether these animals became infected by exposure to cattle or to humans. Parainfluenza 3 spreads readily from person to person so that bovine or other animal sources are unnecessary to explain frequent human infections. However, the possibility that domestic animals may serve as reservoirs of common human viruses requires careful study.

Interest in the problem of respiratory disease of cattle is international. During their research, Dr. Abinanti and his co-workers in the Laboratory of Infectious Diseases have run serological tests of bovine sera not only from different parts of the United States but from Tahiti and Japan, and have demonstrated parainfluenza 3 antibody in these sera. In Sweden, investigators at the Swedish Institute of Veterinary Medicine have reported isolation of a virus identical with parainfluenza 3, which they believe might be the primary cause of so-called transport disease.

VARIANTS RESIST TEMPERATURES PREVIOUSLY THOUGHT TO DESTROY ALL THREE POLIOVIRUSES

A research team supported by an NIAID grant revealed poliovirus variants which resisted temperatures generally believed to be rapidly destructive of all three types of virus. Drs. Donald N. Medearis, Jr., John H. Arnold and John F. Enders conclude in the Proceedings of the Society for Experimental Biology and Medicine that survival of appreciable quantities of poliovirus under the conditions imposed by their experiments makes it doubtful whether pasteurization of milk and other food products as now carried out can assure completely the inactivation of these agents.

The scientists obtained their results in a series of experiments initiated after unexpected multiplication of poliovirus Type II had been encountered in safety testing of a suspension heated at 65 degrees centigrade. It had been intended to employ this suspension as antigen in skin tests on human beings.

Their experiments showed that suspensions of polioviruses Types I, II, and III, in medium 199 derived from infected monkey kidney cell cultures, were not completely inactivated following exposure for 1 hour to temperatures of 60 degrees and 65 degrees centigrade. In one instance infectious virus was demonstrated in a suspension of poliovirus Type II after heating at 75 degrees centigrade for 1 hour. Progeny of virus surviving at this temperature exhibited increased thermoresistance. Acknowledging the need for supplementary experiments, the scientists say that on the basis of their findings it would seem that these highly resistant variants do not

"breed true" in the sense that their progeny are equally resistant. However, mean resistance of the progeny is increased. This would suggest, the scientists believe, that more than a single gene may determine, in association with environmental phenomena, thermoresistance of the progeny.

Factors to account for the difference between these results and those of previous investigators are not readily apparent. The concentration of extraneous protein in these preparations which might enhance thermostability was as low or lower than that in preparations examined by others. The pH was maintained at neutrality or slightly on the alkaline side where the stabilizing effect of cystine is not critically operative. It is possible that the higher concentrations of virus employed in these experiments together with replicate tests in a fairly large number of cell cultures of high sensitivity observed over long periods may be responsible for the discrepancy, the scientists conclude.

COMPLEMENT FIXATION USEFUL TO CONFIRM CLINICAL DIAGNOSIS OF COLORADO TICK FEVER

Diagnosis of Colorado tick fever by complement fixation can be accomplished routinely in most diagnostic laboratories, according to data presented by Drs. Leo A. Thomas and Carl M. Eklund of the National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana. This test, which is much simpler than any of those previously used, is described in the Journal of Infectious Diseases.

Previous studies from the Rocky Mountain Laboratory have shown that Colorado tick fever virus can be isolated readily from blood obtained from patients during the febrile period of their disease, either by inoculation of suckling mice or of KB tissue culture cells, and that isolation and identification of virus is the most rapid method of laboratory diagnosis. These and other research procedures, however, are not suitable for the average diagnostic laboratory.

Drs. Thomas and Eklund, using a complement-fixing antigen prepared by Casal's technique from suckling mouse brains infected with Colorado tick fever virus, found the CF test satisfactory in detecting antibodies in serums from patients convalescent from Colorado tick fever. Complement-fixing antibodies first appeared 20 days after onset of illness, one week later than neutralizing antibodies, and were present in one patient for as long as 260 days.

Acute and convalescent serums were available from more than 50 patients on whom a clinical diagnosis of Colorado tick

fever had been made, substantiated by isolation of virus from blood taken during the acute phase of illness and demonstration of neutralizing antibodies in blood taken during convalescence. Approximately 80 percent of those collected after the fourth week of illness contained demonstrable CF antibodies. The authors conclude that the complement fixation test can be used successfully to detect antibodies following infection with Colorado tick fever virus. They emphasize that serums for the test should be collected 4 weeks or longer following onset of illness. The test should prove of value, they believe, in general diagnostic laboratories as confirmation of the clinical diagnosis of the disease.

STUDIES OF MOUSE POLYOMA (TUMOR) VIRUSES EXTENDED BY FIELD WORK IN NEW YORK CITY

The ability of the mouse polyoma virus to produce multiple tumors in mice and other rodents has aroused great interest and stimulated extensive studies of virus by many laboratories. One of the basic questions raised in relation to this virus is, "What manifestations does the polyoma virus produce as it occurs naturally?"

Drs. Wallace P. Rowe, Robert J. Huebner, and Janet W. Hartley of the National Institute of Allergy and Infectious Diseases reported at the Gustav Stern Symposium on Perspectives in Virology on studies of the natural occurrence of polyoma virus in mice (Mus musculus) inhabiting densely populated areas of New York City, particularly Harlem. The NIH scientists were aided in the field work by the New York City Health and Sanitation Department. Approximately 1,000 mice were studied. The natural history, the importance of ecto-parasites, the mode of spread of the virus, and the tumorigenic activity of "wild" polyoma virus strains were studied. The basic tool for these studies is the hemagglutination inhibition test, which serves both as the indicator of past infection and the end-point of sensitive virus detection tests in laboratory animals and tissue culture systems.

In the Harlem area infection was found in high frequency along certain blocks, while nearby blocks were completely negative. There was marked difference in frequency of infection of mice trapped in different apartment houses within a single block. Virus was recovered from the organs of 14 wild mice, and also twice from floor sweepings in apartments housing infected mice. Virus was isolated from the excreta of three of the mice. Mites recovered from infected mice were found negative for the virus. Cockroaches trapped in the apartments were also negative.

Excretion of virus apparently plays an important role in its transmission in nature. In laboratory experiments, infected newborn mice have been found intense sources of environmental contamination. Eating and drinking contaminated food and water appeared to be possible modes of entrance of virus into mice. However, inhalation of virus appears to be a more effective mode of entry.

The important question of the relation of polyoma virus to the occurrence of spontaneous mouse neoplasms was also discussed at the Gustav Stern Symposium by Dr. Rowe. He described studies undertaken with the collaboration of the Roscoe B. Jackson Laboratories, Bar Harbor, Maine, where large numbers of mice of various inbred strains and hybrid combinations are observed for life and autopsied when moribund from any cause. Polyoma antibody in tumorous mice was compared with that in mice dying without tumors. Among the inbred strains there was not the slightest indication of any excess of polyoma antibody among tumorous mice compared with comparable non-tumorous mice. In the hybrids the situation was essentially the same.

VIRUS MAY BE UNNECESSARY TO MAINTAIN TUMOR IN VIVO ONCE TUMOR INITIATED BY POLYOMA

Recently completed studies by Dr. Karl Habel, Chief of NIAID's Laboratory of Biology of Viruses, and his associates, on the factors responsible for the tumor-producing properties of polyoma viruses have raised a question concerning the necessity for virus presence in maintenance of the tumor. Findings were reported at the meeting of the Federation of American Societies for Experimental Biology, 1960.

Dr. Habel inoculated newborn mice and hamsters with polyoma virus to produce fibrosarcomas which were transplanted into adults of the corresponding species. In the hamster, although virus could be demonstrated in the original tumor, no further evidence for its presence could be found after the first transplant. Attempts to induce these virus-negative tumor transplants to produce virus in tissue culture under X-ray or ultraviolet radiation failed. On the other hand, in one series of mouse tumor transplants the association of virus with tumor has been consistently positive for 10 transplants.

When virus was given to newborn hamsters, along with antiviral serum at a different site of inoculation, tumors appeared after a longer incubation period. Certain of these tumors apparently were not producing virus, however, since the animals gave no evidence of harboring virus antibodies.

The investigators feel that continuing studies of this nature may help clarify tumor-producing properties in other agents and may eventually lead to better understanding of tumor development under natural conditions in man.

ENCEPHALOMYOCARDITIS VIRUS NOW IMPLICATED IN ANIMAL DISEASE AS CAUSE OF DEATH OF SWINE

A group of scientists has for the first time described a natural infection of swine with the EMC virus. Dr. Thomas G. Murnane of the U. S. Army Mission to Panama, Drs. John E. Craighead and Alexis Shelokov of NIAID's Middle America Research Unit, and Dr. Harold Mondragon of the Gorgas Hospital, reporting in Science, believe it is the most extensive outbreak of EMC infection either in man or in animals, in which the virus has been recovered. In all cases, the investigators found severe myocarditis to be the outstanding lesion.

The outbreak of disease occurred on a commercial swine farm near Panama City where approximately 30 pigs died during a 20-day period in one feedlot of 300 animals. Three of the animals were examined histologically, revealing severe myocarditis with round cell infiltration, vascular congestion, edema, and degeneration of the myocardial fibers; mild pneumonitis and pulmonary edema; mild meningitis and minimal congestion of the brain with spotty areas of neuronal degeneration.

As a result of bacteriological and virological studies performed on two of the animals by the investigators, an agent pathogenic for mice was repeatedly isolated from the lung and spleen of the animals and from one of the brains. The same agent was recovered from the brain, lung, and spleen of inoculated mice.

EMC virus is also pathogenic for young hamsters and guinea pigs, and has been re-isolated and passaged in cell cultures of rhesus monkey kidney, hamster kidney, and HeLa cells.

The scientists identified the agent by neutralization tests in mice and tissue culture with a hyperimmune rabbit antiserum prepared against the American Type Culture prototype strain of EMC virus. Identification was confirmed at the Walter Reed Army Institute of Research.

The disease was successfully reproduced by inoculation of 2 young pigs with mouse-passage virus. In both animals viremia was demonstrated on the second day with the first signs of disease appearing on the third. As in the naturally infected animals, the scientists found a severe myocarditis upon sacrifice or death.

AEDES VEXANS MOSQUITO IMPLICATED IN EASTERN EQUINE ENCEPHALITIS IN CONNECTICUT

Supported by a grant from the National Institute of Allergy and Infectious Diseases,

Dr. R. C. Wallis and associates of the Yale University School of Medicine have for the first time isolated EEE virus from the Aedes vexans mosquito. The report is published in the Proceedings of the Society for Experimental Biology and Medicine.

In their study the scientists took weekly samplings of mosquitoes during the spring, summer and early fall of 1959, at the Shade Swamp Fish and Game Wild Life Refuge in Farmington, Connecticut, where epidemics of EEE occur irregularly among the pheasant population. Adult female mosquitoes from these samplings were stupified with cigarette smoke, sorted into pools by species, stored and later processed in the virus laboratory.

Of 3,194 mosquitoes collected, 70 percent were of the Aedes cinereus and canadensis species. Although Aedes vexans accounted for less than 5 percent of the specimens, the only virus isolation was made from a pool of 13 females of this species. Identification was made by both hemagglutination-inhibition and neutralization methods by titrating against a known EEE immune serum.

Dr. Wallis indicates that circumstantial evidence had long supported the possibility of EEE transmission by mosquitoes. In 1956, during an encephalitis epidemic in pheasants in New Jersey, four isolations of EEE virus were obtained from pools of Culiseta melanura, providing strong evidence that this species was a significant vector in birds. Correlation of this species' distribution with incidence of the disease could not be confirmed in Connecticut despite several studies during which, however, A. vexans was found repeatedly. Unlike C. melanura, its distribution extended to all areas where virus activity occurred. This epidemiological consideration, plus the knowledge that A. vexans is a vicious biting mosquito; has a wide variety in its host range, feeding readily on man, horses, cattle and birds; enters houses and barns and attains its population peak late in the summer just prior to the EEE epidemic season in the New England States, led investigators to consider it as a probable vector. With the present isolation of the virus from A. vexans, this consideration has been confirmed and A. vexans assumes a new epidemiological significance.

MOSQUITOES, VIRUSES IMPLICATED IN NEW HUMAN EPIDEMIC HEMORRAGIC FEVERS

Several previously recognized types of virus and others closely related--some observed for the first time--apparently have an etiologic role in a new and frequently fatal type of hemorrhagic fever occurring in the Philippine Islands and in Thailand. Drs. W. McD. Hammon, A. Rudnick, and G. E. Sather of the University of Pittsburgh have reported epidemiologic, clinical, and etiological studies on this highly lethal disease in Science. The work was supported by a grant from the National Institute of Allergy and Infectious Diseases.

The scientists reported the occurrence of approximately 3,250 cases in Manila and Bangkok, 10 percent of which were fatal. Nearly all patients were children below the age of six from areas where Aedes aegypti mosquitoes were present in large numbers. The clinical manifestations of the disease varied somewhat in the two locations, leading the scientists to postulate the presence of two or more related but different agents.

Mosquitoes were collected from the epidemic area and serum specimens were obtained from patients in the acute and convalescent phases of the disease. In the Manila area, 14 viruses were isolated by adaptation to suckling mice, and significant rises in antibody titer to those viruses were demonstrated in several cases in the epidemic. All of the viruses isolated during the epidemic in Manila proved to be antigenically related to dengue, types 1 and 2, but represented newly recognized viruses for the most part. These were provisionally designated as dengue, types 3 and 4.

Human sera yielded dengue virus types 2,3, and 4, while Aedes aegypti and Culex tritaeniorhynchus yielded dengue, type 3. This was the first instance ever reported of the isolation of a dengue virus from wild-caught mosquitoes.

Of nine virus isolations from the epidemic in Bangkok, six were antigenically similar to dengue type 2, the remainder to chikungunya virus (a group A arthropod-borne virus known to cause a dengue-like febrile disease in Africa). Three viruses were isolated from A. aegypti and characterized as dengue, type 2, while all other dengue and chikungunya viruses were isolated from serum specimens.

Serological studies, as yet incomplete, have related infection with one or more of these viruses to the disease by demonstrating a rise of antibody titer in suitably spaced paired specimens.

In summary, although the investigators feel it is premature to asscciate the presence of all these agents in the blood of patients suffering from the broad clinical syndrome of hemorrhagic fever with the A. aegypti mosquitoes of the two epidemic urban areas, the bulk of evidence points to a probable etiologic relationship.

NEW VIRUS OF ARTHROPOD-BORNE GROUP B ISOLATED FROM COLORADO TICKS

From a pool of 20 ticks collected along the North Cache la Poudre River, Colorado, a virus has been isolated at the Rocky Mountain Laboratory which has been shown to be closely related if not identical to Powassan virus. In 1958 Powassan virus was found in the western hemisphere for the first time. A group of Canadian workers isolated it from the brain of a 5-year-old boy dead of an acute encephalitis.

The isolation at the Rocky Mountain Laboratory is reported in the Proceedings of the Society for Experimental Biology and Medicine by Drs. Leo A. Thomas, Richard A. Kennedy, and Carl M. Eklund of the National Institute of Allergy and Infectious Diseases. Isolation in the United States of an agent related to a group of viruses known to cause extremely severe illness in other parts of the world necessitates continued studies to determine the ecological factors associated with the virus and the degree to which man is exposed to infection, the authors believe.

Ticks (Dermacentor andersoni) were injected into mice which then developed illness suggestive of encephalitis. Serologic studies of the agent isolated from the mice included hemagglutination, hemagglutination-inhibition, complement fixation, and neutralization tests. These tests differentiated the virus from others such as Japanese B, Ilheus, Murray Valley, West Nile, St. Louis, and Russian spring-summer encephalitis viruses, but not from Powassan virus.

Scientists at the RML have studied ticks in the western United States for a number of years to determine their role in disease. Techniques suitable for isolation of the Powassan-like agent have been employed and many isolations of tick fever virus made from thousands of ticks, but until the present study there was no evidence that other viruses were present. According to the investigators, these studies suggest that some insect other than ticks may be a reservoir or vector of the virus. There have been some observations based on serologic studies that certain individuals in the Western States have antibodies against group B arthropod-borne viruses which cannot be accounted for by the agents previously known to be present in these areas.

ALLERGY—IMMUNOLOGY

SITE OF INTRACELLULAR ANTIGEN DEMONSTRATED BY FLUORESCENCE IN THREE MYXOVIRUSES

The finding that intranuclear antigen production so prominent in the multiplication cycle of influenza and fowl plague viruses is not demonstrable in the cycle of Newcastle disease virus, Sendai, or mumps viruses is reported by Drs. Myrna I. Traver, Robert L. Northrop and Duard L. Walker of the University of Wisconsin. The report of this work, supported partly by a grant from the National Institute of Allergy and Infectious Diseases, appears in the Proceedings of the Society for Experimental Biology and Medicine.

Using fluorescent antibody technique, the investigators compared the multiplication cycles of NDV, Sendai and mumps viruses with that of influenza A virus. They used antisera from three species, several multiplicities of infection, and two cell types to assure that the observed patterns of antigen development and distribution were not peculiar to a special set of materials or conditions. For examination by fluorescence microscopy cells were grown on cover glasses in tubes with flattened sides. Controls consisted of uninfected cells treated with specific conjugate, infected cells treated with normal serum, and on certain occasions, infected cells pretreated with unlabeled antibody prior to staining with labeled antibody.

The three non-influenza viruses (NDV, Sendai, mumps) were observed through an entire cycle of infectivity. During periods up to 26 hours, nuclei appeared consistently as clear, non-fluorescent areas; specific viral antigen was visible in the cells as granules distributed throughout the cytoplasm. A marked difference was observed with influenza virus. In this virus, antigen was first seen by fluorescence in the nucleus, followed by later appearance in the cytoplasm.

The authors emphasize that while the present study indicates that intranuclear production of antigen demonstrable by fluorescent antibody is not characteristic of the non-influenza viruses tested, it remains possible that the nucleoprotein antigen is produced within the nucleus in a form that does not combine with antibody under the conditions of current fluorescent antibody technique. On the other hand, it may be that with these viruses antigen is produced not in the nucleus but in the cell cytoplasm. Studies are in progress to clarify this point. The demonstration that intranuclear antigen production so prominent in the multiplication cycle of influenza and fowl plague viruses is not demonstrable

in the cycle of NDV, Sendai and mumps viruses may add another characteristic to those which seem to separate the myxoviruses into two distinct subgroups.

NEW DIAGNOSTIC PROCEDURE DEVELOPED FOR LUPUS ERYTHEMATOSUS

A simple and rapid diagnostic test for lupus erythematosus has been developed by scientists of the National Institute of Allergy and Infectious Diseases. Mr. John Bozicevich of the Laboratory of Immunology, in cooperation with Drs. John P. Nasou and Donald E. Kayhoe of the Laboratory of Clinical Investigation, describe the procedure as similar to the bentonite flocculation test originally devised against rheumatoid arthritis.

In this case, however, desoxyribonucleic acid (DNA) is substituted for gamma globulin as the sensitizing agent for the bentonite particles. These are added to heat-inactivated serum dilutions to effect the flocculation reaction. The results of the study are reported in the Proceedings of the Society for Experimental Biology and Medicine.

In the clinical evaluation of the new diagnostic procedure in eight patients with lupus erythematosus, the investigators compared the results of the new flocculation test with those of the widely used lupus erythematosus cell test and found them comparable.

The scientists indicate that one of the disadvantages inherent in the L.E. cell test is the need for employing fresh whole blood. In the newly developed DNA-bentonite flocculation test only the patient's serum is required.

While it is true that cases in remission are apparently more sensitive to the older test (two out of five patients of this type yielded positive reactions to the L.E. cell test but reacted negatively to the flocculation tests) the new procedure appears to be more specific. This was indicated in tests with serum from six rheumatoid arthritis patients which gave positive reactions with the L.E. cell test and negative reactions with the flocculation procedure.

As controls, the investigators tested sera from 138 individuals, including normal subjects and those with related and unrelated diseases. The new flocculation procedure gave negative readings in each case. Ninety-four of these 138 individuals were evaluated by the L.E. cell method. Six of them reacted positively even though there was no clinical evidence of the disease.

NEWBORN INFANTS SHOWN CAPABLE OF DEVELOPING SIGNIFICANT ANTIBODIES TO VARIOUS VIRUSES

A recent study published in Pediatrics by Dr. Heinz Eichenwald and Olga Kotsevalov, grantees of the National Institute of Allergy and Infectious Diseases, has cast doubt on the belief that human infants do not begin to form adequate circulating antibodies to injected bacterial antigens until the second month of postnatal life.

Over a 5-year period the scientists tested more than 4,000 infants to obtain sufficient data on the 35 infants illustrating their report. Natural infections studied included those resulting from adenovirus types 1, 2, and 3 and ECHO virus types 9, 18, and 20. Standard serological techniques were employed in identifying the isolated cytopathogenic agents, and neutralizing antibody titers were determined for each patient by testing serum against the particular strain of virus isolated.

The scientists feel that their data are not sufficiently extensive to warrant many definite conclusions concerning the immunologic responsiveness of premature and young full-term infants to viral infection. Viruses are detectable in these special groups only rarely and then the interval between the time a specimen is obtained and a virus isolated may be too long to permit adequate serologic follow-up.

They concluded from their findings that it is impossible to generalize on the basis of experience with one virus. For example, 12 premature and 5 full-term infants infected with ECHO virus type 18 were ill with diarrhea, while 4 full-term infants (less than 8 days of age) harboring ECHO type 20 did not manifest any obvious symptoms. Yet, infection with either of these viruses apparently produces consistent antibody response. On the other hand, adenovirus type 1 infection which was not associated with any recognized illness in the 5 premature infants investigated and adenovirus type 3 (6 full-term infants ranging from 12 to 37 days of age) which may produce diarrhea in some and no disease in others, did not always produce a detectable antibody response. Despite this, the infection does not disseminate and eventually subsides. Obviously, the ability to form antibodies represents only a small part of the total host response to infection.

According to the scientists, one general fact emerged from the study: newborn and premature infants naturally infected with various types of ECHO viruses and adenoviruses usually respond promptly with antibody formation. Whether or not detectable antibodies develop is apparently closely related to the inherent antigenicity of the agent--that is, to the particular virus involved--rather than to the patient's maturity.

RELATION BETWEEN DELAYED AND IMMEDIATE HYPERSENSITIVITY IMPORTANT ASPECT IN ALLERGY

Laboratory experiments directed toward unraveling the relation between delayed and immediate types of hypersensitivity are in progress at the Rocky Mountain Laboratory, field station of the National Institute of Allergy and Infectious Diseases in Hamilton, Montana. Only recently has it been possible to demonstrate a relation between immediate and delayed types of sensitivity in experimental animals.

It has been suggested that delayed hypersensitivity is a step in the formation of circulating antibody. This raises the question of whether or not delayed sensitivity has a more primitive type of specificity than do Arthus reactions and circulating antibody. This is among the concepts being explored by Drs. S. B. Salvin and R. F. Smith at the Rocky Mountain Laboratory. Their first paper in a series on specificity of allergic reactions appears in the Journal of Experimental Medicine.

The investigators recognize that two contradictions may seem to invalidate the hypothesis that delayed hypersensitivity is a stage in the production of circulating antibody. First is the difficulty of producing circulating antibodies to denatured proteins, such as gelatin, although delayed hypersensitivity develops; the second, the inability to detect delayed hypersensitivity to purified polysaccharides, although circulating antibody may occur. Both of these facts may be explained on the basis that recognition of antibody by antigen in delayed hypersensitivity is directed toward a broad area of the antigen molecule, and in Arthus reactions and circulating antibody toward a more narrow, finite area.

By employing various types of avian albumins and different conjugated proteins prepared from them, it was possible to show that the delayed reaction is produced in guinea pigs in response to a broad general area of the antigen molecule. Activities of circulating antibody, however, are directed by small, specific groupings of the antigen.

When conjugated proteins, such as picrylated hen albumin, are used as antigens for guinea pigs, the animals develop delayed reactions to the protein portion, and antibodies and immediate reaction to the haptenic portion of the complex.

ALLERGIC ENCEPHALOMYELITIS DEMONSTRATED TO HAVE IMMUNOLOGICAL BASIS

A successful transfer of allergic encephalomyelitis has been brought about by the injection of lymph node cells taken from donor rats sensitized to spinal cord into recipient rats pretreated neonatally with normal rat spleen cells. The study was undertaken at the New York University College of Medicine by Dr. Philip Y. Paterson of the Institute's Laboratory of Immunology. Findings were published in the Journal of Experimental Medicine.

Dr. Paterson pretreated newborn rats with pooled, normal spleen cells from splenectomized adult donor rats. Five to 6 weeks later the donor rats were injected with guinea pig spinal cord in adjuvant. Lymph node cells were collected from the donors 7 to 12 days later. These were pooled, washed and injected intravenously into the pretreated, and now adult, recipient rats. The recipients were observed for 6 to 8 days after lymph node cell transfer, then sacrificed, and their brains and spinal cords examined microscopically for neurological lesions. Comparable lymph node cell transfers were performed using control donors injected with guinea pig kidney-adjuvant and control recipients which were not pretreated with spleen cells.

As a result of the transfer of lymph node cells to recipient rats pretreated with spleen cells from the same donors, 14 of 22 recipients were found to have allergic encephalomyelitis lesions after injection of 10 to 156 million viable donor lymph node cells. The number of lesions demonstrable in each of these 14 positive recipients varied from a minimum of 2 to a maximum of 15 and both their number and intensity appeared to be directly related to the number of donor lymph node cells transferred.

Dr. Paterson performed additional lymph node cell transfers using recipients pretreated neonatally with spleen cells from rats other than those providing the transferred lymph cells. Of these a total of 6 of 29 recipients acquired allergic encephalomyelitis lesions. The investigator attributes the transfers to the use of recipients which have acquired immunological tolerance to donor lymph node cells, as a result of the spleen cell pretreatment, and in which, therefore, the donor cells can survive and function longer after transfer.

The successful transfer of allergic encephalomyelitis from diseased to healthy animals by means of lymph node cells alone establishes the role of these cells in the development of this disease and the importance of delayed sensitivity in its pathogenesis. These experimental results may furnish

the means for answering several important questions about the true nature of allergic reactions to tissue antigens.

ARTIFICIALLY IONIZED AIR OF NO VALUE IN THERAPY OF ASTHMA IN HOSPITAL TEST

Study of the therapeutic potentialities of artificially ionized air has been under way since before World War II. A report in 1946 of beneficial effects of airborne negative ions on paranasal sinusitis and vasomotor rhinitis prompted inquiry by other investigators into the influence of ionization in hay fever. Prompt amelioration of symptoms appeared to be the outcome.

With these results in view, Dr. Bernard Zylberberg of the Department of Allergy, National Jewish Hospital, Denver, chose 16 chronically asthmatic men and women, aged 15 to 53, to undergo two 120-minute exposures to ionized air. This collaborative study with Dr. M. H. Loveless, grantee from Cornell University receiving support of the National Institute of Allergy and Infectious Diseases, is described in the Journal of Allergy. With one exception, the patients chosen had remained asymptomatic for at least 3 hours before the test, despite the prohibition of all medication for 12 hours or more prior to the test.

A total of 40 2-hour test periods were evaluated. In 28 instances the patients started the exposure period without symptoms. This clinical pattern was unchanged by exposure to either positive or negative ions. In seven other tests slight or moderate wheezing was already present just before exposure, and symptoms continued unaltered throughout the 120-minute period, regardless of the charge of the ion.

In 5 of the 40 tests, 3 negative, 2 positive, it appeared that mild or moderate wheezing and dyspnea occurred where none had existed prior to the experiment. The authors assume that this might have been the case had no treatment been given, since the ionization had had no demonstrable effect on the symptom-free patients.

The investigators believe that the design of the experiment, essentially double-blind, made it unlikely that results were influenced by psychogenic factors. Their clear-cut findings incline them to discard ionization as therapy for patients with asthma.

GENETICALLY DETERMINED FACTORS MAY PLAY ROLE IN SARCOIDOSIS

A complex hereditary factor may be operative in the pathogenesis of sarcoidosis, according to Drs. Richard K. Merchant and John P. Utz of the National Institute of Allergy and Infectious Diseases, reporting in the Archives of Internal Medicine. While much has been learned about this disease during the half century since its initial description, its etiology and pathogenesis have remained obscure. The significance of genetic factors is suggested from the authors' observations of the disease in a mother and daughter, together with reports in the literature of 73 other cases of sarcoidosis diagnosed in more than one member of 32 families.

In support of their postulate, the authors contend that the occurrence of sarcoidosis in parent and offspring, as in the present example, might naturally be expected in a few cases. Furthermore, although the total number of instances of this disease in more than one member of the same family is small (non-twin siblings, 21; identical twins, 6; and parent-offspring, 6) examples in identical twins appear with unexpected frequency. If chance alone determined the development of sarcoidosis in the six pairs of identical twins reported, one would expect twice this number in non-identical twins. Yet no such reports have been found in the literature. One might also expect many more cases than have been described among non-twin siblings. However, ideal diagnostic criteria have not been fulfilled in many instances in the reported cases of sarcoidosis in more than one member of a family. Moreover, the occurrence of the disease in identical twins is more apt to be reported than its occurrence in other members of a family.

It is concluded that while these findings do not minimize the importance of recent epidemiological and laboratory studies, they do suggest that genetically determined individual constitutional factors may play a role in determining the type or frequency of response that results from exposure to some environmental factor.

PARASITIC DISEASES

MONKEY MALARIA TRANSMITTED TO HUMANS BY BITE OF MOSQUITO

Transmission of monkey malaria to man through the bite of an infected mosquito--an experimental feat which may compel scientists to re-examine long established knowledge on the transmission cycle of this disease--has been reported by the Laboratory of Parasite Chemotherapy, National Institute of Allergy and Infectious Diseases.

This finding directly challenges the concept of malaria investigators throughout the world that types of malaria which infect animals cannot be transmitted to man by the bite of an infected mosquito. Only in the laboratory has it been possible, up until now, to infect man with monkey malaria, and then only by an artificial method: blood inoculation.

Results of the study are summarized in Science, official publication of the American Association for the Advancement of Science. The authors are Dr. Don E. Eyles, Dr. G. Robert Coatney, and Dr. Morton E. Getz, all of the Laboratory of Parasite Chemotherapy.

The authors emphasize that the implications of their findings, suggesting the possibility that there may exist in nature a monkey-mosquito-man cycle of malaria infection, remain matters of conjecture at this time. They also point out that no one can say as yet whether this new knowledge will have any practical effect on the course of malaria eradication in areas of the world where monkeys and other non-human primates are found.

Referring to these considerations, the authors summarize their views as follows: "We believe that research on lower monkey malarias, with particular regard to the possible existence of reservoirs of human infection, or sources of reinfection of man with new strains, is necessary as an essential adjunct to the present malaria eradication program. We expect to study monkey-human malarias in detail and re-examine the entire question of the host-specificity of primate malarias."

The study reported here originated from accidental infections involving Dr. Eyles and an assistant at the Institute's Memphis Laboratory. In the course of a routine study in which large-scale inoculations of monkeys with primate malaria were being carried out, the two investigators developed illness with fever. Malaria parasites were demonstrated in the blood of both. "Presumably," the authors state, "the source of the malaria was mosquitoes infected with monkey malaria, as neither individual had had any recent contact with human malarias."

Blood taken from each of the infected individuals was injected into uninfected Rhesus monkeys. Both animals developed typical infections 8-10 days later. Then, blood from one of the infected workers was then injected into two inmate volunteers at the federal penitentiary in Atlanta. Both developed clinical attacks of malaria but neither exhibited high parasite densities. These infections are still under study.

The two accidental infections at the Memphis Laboratory were followed by two planned and purposeful infections involving

staff members. The first volunteer allowed 30 to 50 Anopheles freeborni mosquitoes heavily infected with monkey malaria to feed on him. Eleven and 12 days later he experienced typical symptoms of malaria infection: headache, malaise, and elevated temperature. When parasites could not be demonstrated, a sample of his blood was injected into an uninfected monkey. Six days later the animal came down with malaria, indicating a low-grade infection of the human subject whose blood had been injected into the monkey.

The second staff member allowed himself to be bitten by 10 mosquitoes heavily infected with the strain of primate malaria under study. Fourteen days later he came down with clinical malaria, and parasites were easily demonstrated in his blood. Both volunteers then received standard antimalarial treatment, followed by uneventful recovery.

The parasite causing this disease is known as Plasmodium cynomologi, subspecies bastianelli, and was recently described by professor P. C. C. Garnham of the London School of Hygiene and Tropical Medicine. The new subspecies was isolated from a Kra monkey from Malaya and infected blood was forwarded to the NIH investigators by Professor Garnham for study purposes.

According to the authors, the findings of this investigation underline the need for immediate and intensive studies of monkey malaria in various parts of the world in order to determine their significance.

FLUORESCENT ANTIBODY TECHNIC USED TO DEMONSTRATE PARASITE OF HUMAN MALARIA

A diagnostic test which may make it possible for a single laboratory worker to screen many more suspects for malaria in a single day was described at the Ninth Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 2-5, by Drs. John E. Tobie of the National Institute of Allergy and Infectious Diseases Laboratory of Immunology and G. Robert Coatney of the Laboratory of Parasite Chemotherapy.

The new test, based on the fluorescent antibody technique, represents the first recorded instance in which a malaria parasite (Plasmodium vivax) has been viewed by this method. As in other tests of this nature, antibodies--in this instance from a serum fraction of a patient harboring the vivax type of malaria--are labeled with a special fluorescent dye (fluorescein isothiocyanate) and the fluorescent antibodies applied to a blood smear containing the malaria parasites. The fluorescent antibody reacts with the parasite, which serves as antigen, so that when the parasites are stimulated with ultraviolet light, they glow. Prior removal of

hemoglobin from the blood smear provides a slide in which the red blood cells are not visible--essentially just the glowing malaria parasites.

To evaluate the new procedure, the scientists tested blood smears from seven inmate volunteers known to harbor the vivax type of malaria parasite. Under the fluorescence microscope, each specimen of the prepared parasite--glowing a bright yellow color--stood out clearly from the rest of the field. Using the same procedure, the investigators were able to visualize the parasite of monkey malaria (Plasmodium cynomolgi bastianellii) with the same clarity of detail.

Serological tests, such as complement fixation, precipitin or agglutination tests for the diagnosis of malaria, essentially have been of no practical value. For such diagnosis, it has been necessary that well-trained technicians spend many hours in the examination of conventionally stained blood smears. With the new procedure, a far more rapid method of screening is within the realm of possibility. The picture presented under the fluorescence microscope is such that it may be possible for a photoelectric cell to detect the glowing parasites. For this reason, the scientists are currently exploring the possibility of feeding slides of individual test specimens serially through an electronically controlled automatic scanning microscope for the detection of parasites in large-scale surveys.

The scientists emphasized that their studies were strictly of a preliminary nature and that many technical difficulties must be solved before their goals may be achieved.

As a part of their study, the investigators studied the serological relationships between a human malaria parasite, P. vivax and a rodent malaria parasite, P. berghei. When viewed under the fluorescence microscope, the former glowed approximately three times as bright as the latter, suggesting a distinct difference between the two species. However, when the human type (P. vivax) was compared with the parasite of monkey malaria (P. cynomolgi bastianellii), both species glowed at essentially the same intensity indicating that they may be closely related malaria parasites.

Despite the relative success of the World Health Organization program to eradicate malaria, nearly half of the world's population continues to live in areas of exposure. Coming at the present time, the new procedure assumes an added significance in view of the further diagnostic difficulties, now being imposed on the already overburdened technician, with the recent discovery that monkey malaria may provide a permanent reservoir for the disease.

FLUORESCENCE OF FILARIAL WORM SUGGESTS NEW TOOL IN DIAGNOSIS OF PARASITIC DISEASES

The discovery by two National Institute of Allergy and Infectious Diseases scientists that filarial worms may be visualized by a simple fluorescent method in a patient receiving tetracycline offers new diagnostic and therapeutic possibilities in the campaign against parasitic diseases. Dr. John Tobie, Acting Chief of the Laboratory of Immunology, and co-investigator Dr. Henry K. Beye of the Laboratory of Clinical Investigation have published a detailed description of the work leading to this finding in the Proceedings of the Society for Experimental Biology and Medicine.

Investigation of the phenomena was based on the knowledge that tetracyclines fluoresce a yellow-gold color when exposed to ultraviolet light, and on their recent study showing that this series of antibiotics is deposited in filarial worms in vitro. The patient under study was a 15-year old female with so-called fugitive swellings on both trunk and extremities. Examination of a blood sample confirmed the presence of unsheathed microfilariae of Acanthocheilonema perstans.

Twenty-four hours after an initial dose of tetracycline (given 1 g. daily by mouth), the patient was placed in a dark room and her skin fluoresced with an ultraviolet hand lamp. Linear, yellow-fluorescent streaks were seen over many parts of the body. When traced at intervals over a 6-hour period, these appeared to migrate in a circular fashion over a 5 to 6-inch area. From these observations, it seemed evident that the worms had selectively taken up a considerably larger amount of drug than the surrounding tissues, which presented only the usual blue-gray autofluorescence of uninvolved skin areas.

According to the scientists, the selective concentration of tetracyclines by microfilariae and adult worms in host tissues may provide two therapeutic possibilities. For one, these antibiotics may themselves have some antifilarial effect, since the treated microfilariae survived less well under ultraviolet stimulation than did the untreated specimens. Second, it may be possible to combine some active antifilarial compound with tetracycline to permit a greater and more rapid concentration of antifilarial drug in worms within the host tissues. Since the tetracyclines can be localized and visualized in worms by fluorescent methods, this might serve as an effective tool in studying the mode of action of these drugs.

TREATMENT OF SCHISTOSOMIASIS WITH DRUG STIBOPHEN GREATLY IMPROVED BY BETTER DIET

A series of nutritional studies on mice may have broad implications in the treatment of human schistosomiasis. According to Drs. George Luttermoser and William B. DeWitt of the National Institute of Allergy and Infectious Diseases Laboratory of Parasite Chemotherapy and Laboratory of Parasitic Diseases, the efficacy of stibophen, a common drug used in treating this disease, is greatly improved when the mice are fed an enriched, purified semi-synthetic diet. The scientists reported their findings at the Ninth Annual Meeting of the American Society of Tropical Medicine and Hygiene.

When the investigators maintained the mice on a "completely" purified diet, the therapeutic value of stibophen given concurrently was raised to a high degree of effectiveness; when the animals were fed a regular commercial pellet, the drug remained relatively ineffective. In one trial the average worm reduction on the semi-synthetic diet was 95 percent, as compared with 12 percent in those mice on a regular commercial laboratory diet. The efficacy of stibophen remained high even when the semi-synthetic diet was made low in protein and other chemical factors such as choline, inositol and vitamin B12. This eliminated the general nutritional status of the animals as a cause of the increased therapeutic value of stibophen and implicated specific factors in the diet.

The treatment of schistosomiasis has been unsatisfactory in spite of the many years spent in searching for better drugs. If the results of recent experimental work done with infected animals may be transferred to the human form of schistosomiasis, improved therapy for its victims may be anticipated.

SMALL AMOUNT OF CHEMICAL EFFECTIVELY CONTROLS SNAIL VECTOR OF SCHISTOSOMIASIS

In many areas of the world the cost of eradicating schistosomiasis in the usual manner is not economically feasible because of the comparatively large quantity of chemical needed for destruction of the snail vector. However, a series of laboratory experiments by scientists of the National Institute of Allergy and Infectious Diseases indicates that very low concentrations of sodium pentachlorophenate (NaPCP) will drastically diminish the fecundity of the snail, Australorbis glabratus, and result in lowered viability of the eggs produced. Results of the investigation by Drs. Louis Olivier and Willard T. Haskins of the Laboratory of Parasitic Diseases were published in the American Journal of Tropical Medicine and Hygiene.

Experiments were conducted using NaPCP concentrations of .05 and .1 ppm (parts per million). Although this contrasts with the 10 ppm usually employed in running water and the customary 5 ppm in still water, striking effects on both fecundity and egg viability were observed.

According to the investigating scientists, the effect was especially impressive when the double action of the chemical, i.e., reduction of snail fecundity plus the weakening of egg viability, was considered additively. For example, in one of the experiments, the recovery of viable eggs on the day the test period started was found to be 14.5 e/s/d (eggs per snail per day). During the subsequent test period the recovery of viable eggs varied from .12 to .78 e/s/d. In another test, .1 ppm of the chemical reduced the production of viable eggs from 11.9 e/s/d before the addition of chemical to from 0 to 1.03 e/s/d during the test period.

When destruction of the adult snails by .1 ppm of the chemical is considered along with the other action, the over-all effect becomes even more striking. Indeed, the viable egg recovery was reduced to .11 and .81 egg per original snail in the above tests. These are, respectively, 132 and 15-fold reductions from the pre-test levels.

All previous field experiments with NaPCP have been designed primarily to kill the adult snails, and the measure of the chemical's efficacy has usually been in terms of adult snail mortality after a short-term treatment with the chemical. The scientists conclude, however, that interference with the reproductive capacity of the snails by the use of very low concentrations of the chemical, even if the adult snails are not destroyed, might reduce or interrupt transmission of schistosomiasis at relatively low cost.

MOSQUITO CONTROL AND MASS CHEMOTHERAPY EXTREMELY EFFECTIVE AGAINST FILARIASIS

An evaluation of the filariasis control program in Tahiti, based on surveys made before and after 5 years of standardized treatment and mosquito control, shows that marked improvement in the control of the disease has been effected during the period under study. The findings are reported by Drs. H. N. March, Jacques Laigret, John F. Kessel and Ben Bambridge of the Institut de Recherche Medicales de la Polynesie Francaise and the Department of Infectious Diseases, School of Medicine, University of California, Los Angeles, in the American Journal of Tropical Medicine and Hygiene. This work received support from a grant of the National Institute of Allergy and Infectious Diseases.

The surveys were made on a variety of the manifestations of filariasis in the male population of Tahiti. Surveys for both microfilaremia and elephantiasis encompassed all the males of 15 districts, 3390 in 1949 and 4262 in 1959 for microfilaremia; also in 1959, 7360 persons were examined for elephantiasis.

In 1949, 38 percent of the study population demonstrated a positive microfilaremia and 7 percent were affected with elephantiasis. Ten years later the comparable percentages were 6.5 and 2.3. The control program responsible for this sharp decrease consisted of mass treatment with diethycarbamazine, plus elimination of mosquito breeding areas within 100 meters of dwellings.

Diethylcarbamazine (Notezine) was administered in 12 monthly doses of 6 mg. per kg. of body weight, with additional treatment to the few with recurrent positive blood films. Drug therapy was well accepted. Persons experiencing lymphangitis attacks often requested medication because they were able to observe at first-hand the benefits of therapy.

No new cases of elephantiasis or hydrocele developed following application of control measures. Another important finding was a drop in cases of elephantiasis from 6.9 percent to 2.2 percent for all males. In the significant laboring-age group (20 to 40), there was a reduction from 5 to 0.6 percent. Total hydroceles were reduced from 9.8 to 3.2 percent. Among males older than 20, acute filarial lymphangitis attacks fell from 36 to 4 percent. In untreated cases, these attacks occur several times a year, often requiring 3 days of bedrest per episode.

Projecting the survey findings to a total rural Tahitian population of approximately 20,000, at least some 500 fewer persons were handicapped by elephantiasis in 1959 than in 1949. In persons over 20, attacks of lymphangitis were reduced from 3,430 in 1949 to 380 in 1959. Conversely, the percentage of that group free from signs of clinical filariasis increased to 49 percent in 1959 as contrasted to 16 percent in 1949. Since no new cases have appeared it is expected that when those now infected die, elephantiasis will cease to exist in those islands were an adequate control program is followed.

EPIDEMIOLOGICAL SURVEY OF STATE DEPARTMENT EMPLOYEES REVEALS NEW FACTS ON AMEBIASIS

Results of a survey of State Department employees reveal a surprising lack of correlation between infection with Entameba histolytica and job absenteeism, weight loss or excessive report of diarrhea and abdominal pains.

The survey was carried out jointly by Drs. Donald E. Kayhoe, Henry K. Beye, and Elizabeth Guinn of the National Institute of Allergy and Infectious Diseases Laboratory of Clinical Investigation; and Dr. George P. George of the State Department. It involved nearly 6000 of the more than 35,000 employees and dependents stationed outside the United States. The scientists reported their findings at the Ninth Annual Meeting of the American Society of Tropical Medicine and Hygiene.

Most of these individuals relied on local facilities for food, water, sanitation and medical care. Frequently their meals were prepared according to the prevailing culinary and sanitary customs. Sixteen percent reported no running drinking water in their place of residence and almost all utilized local household servants.

Each participant submitted a stool specimen which was forwarded to the National Institutes of Health for study. Laboratory findings were correlated with replies to questionnaires to provide a rough measurement of the effects of intestinal parasitism on the health of these individuals during their assignment overseas.

Answers to one of the questionnaires on health status were compared against individuals free of parasites, and those harboring Entameba histolytica or Giardia lamblia. While more of those with E. histolytica believed their health was poorer than usual, little difference was found between the three groups in respect to time lost from work. Diarrhea was commonplace, yet, surprisingly, the sufferers included almost as many harboring the ameba as without. At one time, the presence of the parasite was always assumed to be synonymous with overt symptoms of disease. This is now known to be false.

ATABRINE EMPLOYED AS NEW TREATMENT OF BEEF TAPEWORM INFESTATION

A more effective treatment for beef tapeworm infestation may result from the use of atabrine administered through a duodenal tube according to Drs. Donald E. Kayhoe and Henry K. Beye of the Laboratory of Clinical Investigation. The scientists reported their findings at the Ninth Annual Meeting of the American Society of Tropical Medicine and Hygiene.

The study group consisted of 23 patients including 15 who had been unsuccessfully treated by conventional methods. A concentrated solution of antabrine was administered

directly into each patient's duodenum with a Rheyfus tube whose position had first been verified by X-ray.

Thirty minutes later the patients were purged with sodium sulfate solution via the tube and stools were examined for the head of the tapeworm. Those who did not pass the entire parasite at this time were followed for at least one year and if stools remained negative, they were presumed cured. The regime was successful in 20 of 23 patients studied.

NEW DIAGNOSTIC TEST FOR BOVINE LEPTOSPIROSIS SUPERIOR TO OLDER METHODS

At the present time, leptospiruria may be demonstrated by direct darkfield examination, cultural procedures or laboratory animal inoculations. The darkfield method is limited in requiring the presence of a relatively large number of organisms. Use of cultural procedures requires aseptic collection of urine. Inoculation is time-consuming and expensive because the animals must be maintained over a period of time before results are known. A report in the Journal of Immunology describes a modified urine hemolytic test for diagnosing bovine leptospirosis without some of these limitations. The author of the report, Dr. Leif M. Ringen of Washington University, St. Louis, is a grantee of the National Institute of Allergy and Infectious Diseases.

The procedure described by the scientist is a modification of the hemolytic test reported by Dr. Cox in 1955 for the serodiagnosis of leptospirosis using Leptospira biflexa as the source of the erythrocyte sensitizing substance. In Dr. Cox's test, a genus specific reaction is obtained when complement and the sensitized sheep red blood cells are incubated in the presence of specific antibodies. Dr. Ringen's modification uses a soluble erythrocyte sensitizing substance which is precipitated out of bovine urine during the leptospiruria period.

For comparative purposes, the scientist infected a group of calves experimentally with Leptospira pomona. Urine was collected from these animals at weekly intervals and inspected for the presence of leptospires by direct darkfield examination, inoculation into laboratory animals and by the new procedure. The scientist found that leptospiruria may be demonstrated by animal inoculation before the urine hemolytic reaction becomes positive, or vice versa. However, in the latter stages of the disease, the urine hemolytic test continued to be positive after leptospiruria could no longer be demonstrated by animal inoculation. In one group of 116 urine samples, 14 percent were positive and 36 percent negative by both procedures, 42 percent positive to the modified

test alone, but only 8 percent positive to animal inoculation alone. In other animals tested, leptospiruria could be demonstrated only by the modified test. It was difficult to determine the actual numbers of leptospires that could be detected by this procedure, but positive results were obtained from infected urine diluted as much as 10,000 times.

INTESTINAL PARASITISM CONTINUES IN HOSPITAL DESPITE IMPROVEMENT IN ENVIRONMENT

The high incidence of intestinal parasitism in patients in mental institutions has been attributed variously to crowded conditions and to inherent untidiness among these patients. A unique study designed to determine the possible relationship between housing facilities and parasitism in mental patients reveals that transmission of intestinal protozoa continued despite greatly improved housing and sanitation of the group during the three years of the study.

Dr. Geoffrey M. Jeffery of the National Institute of Allergy and Infectious Diseases Laboratory of Parasite Chemotherapy field station in Columbia, S. C., reports his findings in the American Journal of Hygiene. Prior to removal to modern quarters from an extremely outmoded building, a core group of 110 white female patients ranging in age from 18 to 76 years were examined for intestinal parasites. Nine series of examinations were conducted, 4 during the first year (1954), 3 the second, and 2 the third year. This group was studied for the entire 3-year period and was examined on all 9 occasions. In addition, 199 patients had 1 to 8 examinations, depending on the length of time they remained at the South Carolina State Hospital, site of the study.

The physical environment of the patients was also checked regularly, including gross inspection for fecal contamination of the buildings and exercise yards, plus surface sampling of floors, walls and furnishings by the use of cellulose tape. Twenty-six sites within the building were sampled for examination each time.

The incidence of 7 protozoan species from infected inmates was tabulated. Of these species (E. histolytica, E. coli, I. butschlii, E. nana, C. mesnili, T. hominis, and G. lamblia) only C. mesnili and T. hominis showed a net decrease in incidence between initial and final examinations. Some helminths (hookworm, Strongyloides, Trichuris) were also surveyed, and a small reduction in the percentage of patients infected was observed; of these, Strongyloides stercoralis showed the most pronounced reduction.

A single striking increase in incidence was observed in the protozoon E. nana. With this organism, an initial 19.1 percent incidence rose to 62.7 percent on final examination.

Dr. Jeffery believes his data justify the assumption that transmission of protozoa continued steadily throughout the study. The rate of acquisition of protozoa appeared to lessen, however, during the last year or more of residence in the new quarters. The helminths present a different picture. After the patients were transferred to a modern building, there was no evidence of hookworm transmission, and only 3 cases of T. trichiura occurred which could be attributed to residence in the new building.

The question of persistence of parasitic protozoa, according to Dr. Jeffery, is clouded by the distinct possibility that prolonged occurrence of a given parasite in an individual might represent a number of infections of short duration rather than a single prolonged infection. In extrapolating probable percentages of infections within periods of 5, 10 and 20 years, based on average percent of infection per year, in general the parasite species which apparently persist for the longest periods are those with the highest prevalence at any one time. The persistence of hookworm and T. trichiura infections for the full three-year period in almost all cases is indicative of the normally long persistence of these parasites in untreated populations. In work subsequent to that described in this report, it has been shown in this same group that these parasites continue to persist in the majority of cases for a total of at least 5 years.

INDIRECT HEMAGGLUTINATION EFFECTIVELY DETECTS PARASITIC DISEASE CLONORCHIASIS

The successful demonstration that infection with Clonorchis sinensis can be detected by the indirect tanned-cell hemagglutination (IHA) test adds a new diagnostic tool against clonorchiasis, a liver fluke disease highly endemic in the Far East. This work is reported in the American Journal of Tropical Medicine and Hygiene by investigators Guillermo Pancheco, Dale E. Wykoff, and Rodney C. Jung of Tulane University School of Medicine, whose study was supported by a grant from the National Institute of Allergy and Infectious Diseases.

C. sinensis occurs as a common parasite of fish-eating mammals. It causes human infection in Japan, Korea, and China. The human disease has also been recorded in Chinese inhabitants of the United States, Cuba, and India, and in native Hawaiians. Man acquires the infection by eating raw, inadequately cooked, fresh-water fish. Dried, salted or pickled flesh of infected fresh-water fish is also a source of the disease in man.

Clonorchiasis responds to treatment with oral gentian violet. Given in the early stages, this medication is believed curative, and may be beneficial even in advanced cases.

Four different sources of sera were used in this study: (1) rabbits experimentally inoculated with metacercariae of C. sinensis; (2) human beings infected with the organism; (3) humans infected with a variety of other parasites; and (4) animals experimentally or naturally infected with other parasites.

The IHA test proved sensitive in detecting C. sinensis. Compared to the complement fixation test previously used, less extract (4%) produced a titer 40 times greater in the IHA test. This result is in agreement, say the authors, with trials of similar tests for Echinococcus. Other investigators have used indirect hemagglutination on a variety of parasites, including Schistosoma, Trichinella, Echinococcus and ascarids, with equal effect.

Specificity as well as sensitivity of the IHA test was found to be increased by treating the test material with ether prior to saline extraction.

The relationship between the antigen-antibody systems demonstrated by IHA and by CF has not been determined. The antibody titers found by IHA in experimental infections in rabbits appear to rise early in the infection, reaching a peak between the 6th and 16th weeks and then decreasing. A similar rise and fall of antibody titers has been reported by other investigators who used a CF test.

BACTERIAL DISEASES

NEW SYNTHETIC PENICILLIN PROVES PROMISING AGAINST SEVERE BACTERIAL INFECTIONS

A synthetic penicillin (X-1497) which may be effective against certain forms of resistant staphylococcus has been tested by scientists of the National Institute of Allergy and Infectious Diseases with promising results.

The new drug was first developed commercially by Beecham Laboratories in England. Under the trade name, staphcillin, the United States Bristol Laboratories, which has an arrangement with Beecham, released it to the National Institutes of Health and others for clinical and biochemical tests. Results were presented at a symposium sponsored by the State University of New York, Upstate Medical Center, at Syracuse, to bring together available knowledge concerning the new drug.

In discussing its biochemical aspects, Dr. Harry Steinman of the Laboratory of Clinical Investigation observed that the new synthetic, in contrast to penicillin G (the form of the drug commonly employed against infection) was almost completely resistant to destruction by penicillinase (a staphylococcal enzyme whose production is stimulated in the presence of penicillin). Yet, paradoxically, staphcillin is 10 times as active in inducing penicillinase production in resistant staphylococcus.

In the clinical trials, Drs. David Rifkind and Vernon Knight of the same Laboratory administered the drug to 10 patients with a variety of severe staphylococcal and streptococcal infections. Typical among these patients was a 27-year-old housewife who had been treated for staphylococcal bloodstream infection with tetracycline, penicillin G, chloramphenicol and erythromycin, without success. Twenty-one days of therapy with penicillin X-1497 resulted in an uneventful and sustained recovery.

Another patient, a 9-year-old male, had suffered recurrent staphylococcal infections for most of his lifetime. These included, among others, 10 episodes of pneumonia, 4 of bloodstream infection and innumerable abscesses. Thirty-five days of therapy resulted in the disappearance of fever and healing of all skin lesions. Cultures derived from the nose and liver no longer harbored the organism. As with the above, all other cases of infection were followed by recovery or other evidence of effective antimicrobial activity after treatment.

The scientists commented that the present difficulties in the treatment of severe staphylococcal infections are derived largely from the fact that many cultures are resistant to the older penicillins, making even huge doses of drugs ineffective. Moreover, infections in which large populations of staphylococci are present, even though susceptible, may respond poorly to treatment with these drugs. In contrast, penicillin X-1497 has thus far proven active against all strains of staphylococci tested, even those that are resistant to the older penicillins. And finally, the new penicillin has been found to be active against large populations of staphylococci.

In a separate study conducted by Dr. Richard Aster of the National Institute of Arthritis and Metabolic Diseases, results suggest an immunological difference between penicillin X-1497 and other penicillins and indicate the possibility that the new drug may be tolerated by those individuals allergic to penicillin G. The investigator tested serum specimens from one of the cases for antibodies against penicillin X-1497. Although antibodies were noted for other penicillins, presumably from previous treatment, none was found in response to the new synthetic drug.

NEW VARIABLE OF STAPHYLOCOCCUS EPIDEMICS IN HOSPITALS IS CONCOMITANT VIRUS INFECTION

"Cloud Babies," infants who showed no overt sign of disease but assumed highly infectious roles in hospital nursery staphylococcus epidemics, are described for the first time in a study supported by the National Institute of Allergy and Infectious Diseases. Respiratory viruses, which operate independently of staphylococcus, appeared responsible for the high infectiousness of the "cloud baby" in a number of nursery epidemics reported by Dr. Heinz F. Eichenwald and associates in the American Journal of Diseases of Children.

The authors point out that although neonatal staphylococcus is a substantial cause of death in infancy and consequently has received much attention throughout the world, the complexity of modern medical institutions has made it a difficult disease to study. However, their detailed studies on the spread of staphylococci in a number of different nurseries at the New York Hospital-Cornell Medical Center showed that newborn infants infected with staphylococci may fall into one of two distinct groups.

The majority of babies examined possessed a low index of infectivity, while a small minority were highly infectious. Because infants of the latter group were literally surrounded by clouds of bacteria, they are designated "cloud babies" in the report. Follow-up studies showed that their explosive role in the hospital epidemics continued, after discharge, within the family unit.

The bacteriologic factors that determine whether any given infant becomes a "cloud baby" were investigated by use of air-samplers and epidemiologic techniques. The clinically well "cloud baby" contaminated the atmosphere chiefly from the respiratory tract, with skin, cord, or clothing representing only a minor source of microbes.

The investigations also showed that a factor is responsible for the "phenomenon of cloudiness" which operates independently of the staphylococcus, is itself infectious, and has a distinct epidemiology of its own. This factor appeared to consist of a number of respiratory viruses occasionally encountered in hospitalized newborn infants. Adenovirus Type 2 and ECHO virus Type 20 were isolated from two of three test groups.

During the observations it was possible to compare the degree of infectivity of nondisseminating staphylococcal carrier babies and "cloud babies." Two carrier babies were transferred into a nursery that had been under close observation for several weeks and in which the number and identity of infected infants and staff were known. During a 4-day stay

by the carrier babies and for 4 days thereafter no contamination of the nursery air could be demonstrated, the staff continued to have negative cultures, and only 1 of 41 infants in the nursery became infected with Staphylococcus aureus Type 80/81.

Following this determination, another baby was transferred into the unit. He was removed after a 2-day stay when it was discovered that he was a "cloud baby;" nevertheless, colonization rates among the infants increased within 24 hours after his arrival. Within a few days about one-half of the babies had been converted to carriers of S. aureus Type 80/81. Five days after his departure, other "cloud babies" were found in the unit. The presence of these new "cloud babies" was paralleled by a rise in the number of staphylococci Type 80/81 in the air of the nursery and by the appearance of nasal carriers of the organism among the staff. This comparison was again demonstrated in a subsequent controlled situation set up by the scientists.

The report does not minimize the importance of the adult carrier as a source of infection. Though the exact condition under which adult carriers infect infants is unknown and the transmission route has not been clearly established, obviously both staphylococci and viruses must be acquired from someone. It is probable that in ventilated nurseries of adequate size, designed for four or five babies, hospital personnel are of obvious significance, while under conditions of overcrowding, the "cloud baby" may assume a greater relative importance in the genesis of epidemics.

The explosiveness of the "cloud baby" outbreaks, the authors say, forces consideration of airborne dissemination in the design of nursery units. Forced ventilation with frequent air changes, as well as the reinvestigation of ultraviolet light as an additional method of air sterilization seems to the authors the greatest protection against airborne spread.

The authors also note that all carriers of S. aureus Type 80/81 in the study continued to harbor the organism despite novobiocin therapy, whereas those babies who received the antibiotic prior to infection were almost uniformly protected.

EARLY DETECTION AND TREATMENT OF ASYMPTOMATIC BACTERIURIA AVERTS URINARY TRACT INFECTION

Development of a reliable test for the detection of early cases of pyelonephritis was prompted by the finding at autopsy of a 10 to 20 percent incidence of that disease, which is often unsuspected or misdiagnosed. Dr. Edward H. Kass

of Harvard University described at the Chicago Medical Society annual clinical conference a study of the relationship of bacteriuria to serious kidney disease. Dr. Kass has also published his findings in the A.M.A. Archives of Internal Medicine. This work received grant support from the National Institute of Allergy and Infectious Diseases.

To perform the simple test, urine specimens are collected under reasonably antiseptic conditions. Bacterial colony counts on the fresh specimens are then made. Counts greater than 100,000 per milliliter generally indicate the presence of true bacteriuria, which is actual multiplication of bacteria within the urinary tract. Two separate counts are made before the test is considered diagnostic. Three-fourths of infected patients respond to treatment with sulfonamides (0.5 gm. daily for one or two weeks). Alternative therapy on the basis of sensitivity studies is used for patients resistant to sulfonamides.

Dr. Kass has observed that pyelonephritis of pregnancy occurs almost exclusively in patients who have bacteriuria at the time of the first prenatal visit. The way is now clear, with this test, for the virtual elimination of pyelonephritis among pregnant women.

In a group of 90 pregnant women at Boston City Hospital, patients with asymptomatic bacteriuria who were less than 8 months pregnant were divided into two groups on an alternating basis. One group received a lactose placebo, the other was treated until term. Incidence of pyelonephritis in the control group was 42 percent. There was no symptomatic infection in patients whose bacteriuria had been eliminated by treatment.

Follow-up data have been obtained 3 to 12 months post partum. Incomplete results to date indicate that about three-fourths of the untreated patients were still infected 3 months after delivery but more than three-fourths of the treated patients were free of bacteriuria when studied 3 to 12 months post partum.

Based on the observation that 30 percent of female and 10 percent of male patients have pyelonephritis, Dr. Kass suggests that every medical patient be considered a potential candidate for this disease. It occurs widely not only in pregnant women but in persons suffering from obstructive diseases of the urinary tract, diabetes mellitus, hypertension and uremia, and is also one of the commonest causes of acute renal failure.

PENICILLIN REDUCES MORTALITY IN EXPERIMENTAL STAPHYLOCOCCOSIS UNDER TEST CONDITIONS

Results of a study reported by grantees of the National Institute of Allergy and Infectious Diseases may help to define the disputed value of penicillin against infections caused by penicillin-resistant staphylococcus. Drs. Donald B. Louria and David E. Rogers conclude, in a report in the Journal of Laboratory and Clinical Medicine, that penicillin can under certain circumstances significantly reduce the mortality resulting from infection produced by injecting large numbers of penicillin-resistant staphylococci into mice. The most important circumstances affecting the outcome of treatment are the amount of penicillin employed and the time therapy is begun.

In a series of experiments employing a highly virulent strain of staphylococcus, the scientists found that the total mortality in untreated mice ranged from 80 to 100 percent, with only 4 of 117 mice surviving the experimental period. When penicillin treatment was started within 4 hours after infection, survival was prolonged and rates of mortality reduced. For example, in mice receiving 4,000 to 10,000 units of penicillin daily, although death was delayed, at least 75 percent of the mice in each group eventually died. When 40,000 units of procaine penicillin or 100,000 units of aqueous penicillin were administered the mortaility was reduced to 50 and 25 percent respectively.

Delaying treatment 24 to 48 hours after infection markedly reduced the beneficial effects of penicillin. Survival rates varied from 0 to 25 percent and did not correlate well with the amount of penicillin administered. In animals receiving early treatment, survival rates ranged from 3 to 75 percent and were well correlated with the quantity of penicillin injected. When treatment was begun 4 hours after infection, at least 45 percent of the mice receiving 20,000 units of penicillin twice daily survived. In contrast, prolonged survival was uncommon in animals receiving delayed treatment regardless of the dosage employed, although death was often significantly delayed. Thus, both the dose employed and the time at which therapy was initiated had a significant effect on the outcome of the penicillin-resistant staphylococcal infection.

Neither the time of administration nor the dose of penicillin employed appeared to be of critical importance in the treatment of a similar infection in mice produced with a penicillin-susceptible strain of staphylococci. Virtually all treated animals infected with the penicillin-sensitive strain survived whether treatment was started 4 or 24 hours after infection.

TIME-SAVING NEW TEST DETERMINES POTENTIAL PATHOGENICITY OF CLINICAL STAPHYLOCOCCUS

An observation that potentially pathogenic staphylococci may be differentiated from harmless strains by their ability to reduce tellurite underlies the successful development of a new and reliable test by scientists of the University of Utah College of Medicine and the Clinical Microbiology Laboratory, Salt Lake County General Hospital, Salt Lake City, Utah. Dr. Hoeprich and associates, who published the results of the study in the Journal of Laboratory and Clinical Medicine, are supported by a grant from the National Institute of Allergy and Infectious Diseases. The data presented in the grantees' paper indicate that testing for tellurite reduction provides information of the same significance as testing for coagulase production.

Ability to reduce tellurite is quite simply assayed. Isolates to be tested are streaked on a sector of a tellurite-glycine agar plate; 8 tests are conveniently done on 1 plate. After overnight incubation the plates are inspected for the presence of jet black colonies indicative of growth with reduction of tellurite. With periods of incubation much in excess of 18 hours, some staphylococcal isolates which show no growth, or at most a grayish to brownish smudge in 18 hours, will develop black colonies. Not only is tellurite reduction conveniently carried out, but the cost per test is quite small--about one-half cent per test when 8 isolates are inoculated per plate.

DIRECT EVIDENCE PRESENTED THAT PENICILLINASE IS INDUCIBLE ENZYME OF STAPHYLOCOCCUS AUREUS

Further evidence to support the contested view that penicillinase is an inducible enzyme of Staphylococcus aureus was cited at the 1960 meetings of the Federation of American Societies for Experimental Biology, in a paper read by Dr. Harry Steinman of the National Institute of Allergy and Infectious Diseases Laboratory of Clinical Investigation.

In this study Dr. Steinman used an organic mercury derivative to inactivate the bacterium without affecting its penicillinase activity, thus permitting exact analysis of the enzyme. Enzyme activities were demonstrated by testing samples removed from a reaction mixture at periodic intervals. Curves plotted from these data were similar to those obtained by continuous observation of the same reaction system.

The greater accuracy obtained by this method allows more precise evaluation of the experimental curves. Thus the formation of new enzyme was apparent in a synthetic medium

within 15 minutes after exposure of the organism to penicillin and the rate of increase was linear with time. This method also permitted studies on variation of the inducing concentration of penicillin, without interference by the high levels of penicillin required in the subsequent assay procedure. In this way it was established that very high concentrations of penicillin were required for this reaction by the particular strain of S. aureus examined.

More direct evidence for the induced formation was obtained from studies on cell-free preparations wherein concentrated suspensions of S. aureus broken up by bombardment with fine glass beads were found to possess good penicillinase activity.

By means of adsorption and elution of the cell-free enzyme preparation, and fractional precipitation with ammonium sulfate, followed by isoelectric precipitation--a method not hitherto applied to staphylococcal penicillinase--concentration and partial purification of basal penicillinase from organisms not exposed to penicillin was achieved. The increased activity obtained in cell-free extracts from penicillin treated cultures persisted through the purification procedures, indicating that new, induced protein had been actually formed. However, the preparations were still relatively impure since the specific activities were not identical with each other.

A comparison of the physicochemical properties of partially purified penicillinase from S. aureus with purified penicillin from B. cereus indicates that the two enzyme proteins are not identical.

COMBINATION OF NOVOBIOCIN AND TETRACYCLINE FAILS TO DEMONSTRATE SYNERGISTIC ACTION

A controlled study to determine possible synergistic effect on the antibacterial activity of human serum resulting from the combined administration of novobiocin and tetracycline has revealed only additive or indifferent results. Drs. Hans A. Hirsch and Maxwell Finland report this finding in the New England Journal of Medicine. Their study was aided by a grant from the National Institute of Allergy and Infectious Diseases.

The investigators gave eight young men four single doses of the antibiotics at 5-day intervals as follows: 750 mg. of novobiocin; 500 mg. of novobiocin in combination with 250 mg. of tetracycline; and 750 mg. of tetracycline. One week later, all subjects received an additional single capsule of 250 mg. of novobiocin. Blood was taken before breakfast and at 2, 4, 6, 12 and 24 hours later. Serum from each blood was separated

and divided into two rubber-stoppered tubes. One set of serum was assayed for antibacterial activity by a two-fold dilution method in broth, using the following four test organisms: Staphylococcus 209P, Streptococcus 98, Staphylococcus 400 and Staphylococcus 195.

In general, novobiocin was more rapidly absorbed than tetracycline, but the levels in the serum resulting from the former fell more rapidly. As a result, the levels at 2 hours were highest when novobiocin activity predominated. When tetracycline activity predominated, the average levels were highest at 4 hours and thereafter. This became particularly obvious at 24 hours, the height at that time being in the same order as the amount of tetracycline in the dose.

These relations were best illustrated in the activity against Staphylococcus 209P, which was somewhat more sensitive to novobiocin than to tetracycline. Here, the average levels were highest throughout after 750 mg. of novobiocin except at 24 hours, when the highest level resulted from 750 mg. of tetracycline. As the ratio of tetracycline to novobiocin in the dose increased, the average peak levels dropped, and the levels thereafter were increasingly better sustained. The observed differences in the peak levels were all significant except between the doses of 250 mg. of novobiocin with and without 500 mg. of tetracycline. All the differences in total activity resulting from the various doses were also significant except those between the doses of 500 mg. of tetracycline combined with 250 mg. of novobiocin and 750 mg. of tetracycline.

These observations suggest that the combined antibacterial activity resulting from doses of novobiocin in combination with tetracycline was predominantly additive or indifferent. Certainly, no synergistic effect was demonstrated, but a possible antagonistic effect against the tetracycline-resistant Staphylococcus 400 resulted in the serum from the administration of 500 mg. of tetracycline together with 250 mg. of novobiocin.

ENHANCED SUSCEPTIBILITY TO BACTERIAL INFECTION AFTER WHOLE BODY X-RAY STUDIED

A series of studies supported jointly by the University of Chicago, the United States Atomic Energy Commission and the National Institute of Allergy and Infectious Diseases has clarified important details surrounding the known fact of increased mammalian susceptibility to infection after whole body exposure to moderate doses of radiation. Drs. C. Phillip Miller, Carolyn W. Hammond and Sonia K. Anderle made their investigations at the Argonne Cancer Research Hospital in

Chicago and published results in the Journal of Experimental Medicine.

In the experiments the scientists randomly distributed young adult mice into groups of 10 to 20, and exposed them to one of the following doses of x-radiation--300, 400, 500 and 600 r (roentgen units). The mice were then challenged with a virulent strain of Pseudomonas aeruginosa.

In the 400 and 500 r series susceptibility to infection increased rapidly up to the third day after irradiation. In the former, it declined thereafter; but in the latter it was maintained until the 11th day. Each series had returned to normal by the 20th day.

Exposure to 300 r caused but slight increase in susceptibility to experimental infection. Exposure to 600 r was followed by a very marked increase, but caused so many radiation deaths that the results of the inoculations are not comparable with the others.

Mice in the 400 and 500 r series were injected via 2 routes, intraperitoneally and intravenously (dorsal tail vein). Those challenged by the latter method displayed a higher susceptibility to infection. The difference was found to be due to the regular appearance of a small focus of infection at the site of injection as a result of leakage of inoculum into the surrounding tissues. Bacterial multiplication occurred in these locations in irradiated mice, but not in unirradiated mice, nor at the site of intraperitoneal inoculation even in irradiated mice.

FUNGUS DISEASES

CRYSTALLINE HEMOLYTIC COMPONENT OF FIRE ANT VENOM HAS ANTIFUNGAL EFFECT

A crystalline hemolytic component of fire ant venom inhibited the activity of 15 of 21 organisms, mostly human pathogens, demonstrating antimycotic action by a lack of growth immediately surrounding a crystal of this material placed in direct contact with the fungi tested.

This research, an extension of studies of others which indicated the antibiotic character of fire ant venom, is reported by Drs. James T. Sinski, George A. Adrouny, Vincent J. Derbes and Rodney C. Jung, National Institute of Allergy and Infectious Diseases grantees working at Tulane University School of Medicine in New Orleans. The investigators caution that their report is not to be interpreted as a study on the antimycotic effects of this material for

future clinical use but should be considered an attempt to broaden methods for characterizing biologically the crystalline material obtained from fire ant venom.

The tropical fire ant (Solenopsis saevissima) introduced into the United States at Mobile, Alabama, about 1920 is not only a growing agricultural problem in the Southeastern United States but a menace to human beings. The sting of this insect is extremely painful and can cause severe allergic reactions, including anaphylactic shock.

In the experiments reported in the Proceedings of the Society for Experimental Biology and Medicine fungi were streaked onto Petri dishes containing a medium of 2 percent glucose, 1 percent yeast extract, 1 percent casamino acids and 2 percent agar in distilled water. All cultures were tested with a single lot of crude crystalline venom. A few cultures were also tested with a recrystallized venom. A small crystal of venom on each seeded plate was incubated at room temperature, and results were recorded when growth was at its maximum. Inhibition of growth immediately surrounding the crystal occurred with the following: Blastomyces, Candida, Cladosporium, Cryptococcus, Geotrichum, Hormodendrum, Microsporum, Nocardia, Phialophora, and Trichophyton.

EMMONSIA CRESCENS, NEW FUNGUS PATHOGEN OF RODENTS, DESCRIBED

When inhaled by its rodent host, a spore of Emmonsia crescens is 3 to 4 microns in diameter. The spores grow to a maximum diameter of 480 microns, 1,000,000-fold increase in volume, which is extremely unusual in the microbiological world. This fungus was described by Dr. C.W. Emmons, Head of the Medical Mycology Section of the Laboratory of Infectious Diseases, and Dr. W. L. Jellison, Senior Parasitologist, Rocky Mountain Laboratory, National Institute of Allergy and Infectious Diseases, during the 2-day Second Conference on Medical Mycology of the New York Academy of Sciences.

Having increased 1,000,000-fold in volume the spores are incapable of further growth and do not reproduce as long as they remain in the animal. In spite of their enormous size, which makes them easy to see with the naked eye, these fungus cells cause little host reaction except physical displacement of pulmonary tissues. After a month, if the cells are removed to a culture tube from the lung, they germinate by sending out dozens of germination tubes. After several months they may die and calcify.

The fungus has been named E. crescens to denote the immense increase in the size of the fungus cell. The disease it

causes in rodents is called adiaspiromycosis to signify the failure of the fungus to disseminate in the animal. In spite of the size of the fungus and its wide geographic prevalence, pathologists and mammalogists appear either to have overlooked it or perhaps have mistaken it for a worm or other parasite. It has not yet been found in man.

Dr. Jellison, collecting and studying material in Europe during the summer of 1959, found E. crescens in Norway, Sweden, Finland, France, Jugoslavia, Africa, and within 50 miles of the Arctic Circle. In Stockholm, examining preserved museum specimens, he found it in a vole trapped 115 years ago. Dr. Jellison has also discovered the fungus in rodents of the United States, Korea and Ecuador.

In 1942, Dr. Emmons found a similar fungus that was frequent in the lungs of rodents in Southern Arizona. This was designated Haplosporangium. Recently it was transferred to a new genus, Emmonsia, by Italian mycologists Ciferri and Montemartini.

PREVALENCE AND ADAPTABILITY OF FUNGAL DISEASE AGENTS DELINEATED

Dr. Chester W. Emmons of the National Institute of Allergy and Infectious Diseases indicated in his Presidential Address before the Mycological Society of America meeting in Stillwater, Oklahoma that "mycologists must continue to take increasing responsibility in the study of the fungi" which cause systemic mycoses. In one recent year mycotic deaths in the United States equalled the number of poliomyelitis deaths and exceeded by one the total number of deaths reported for whooping cough, diphtheria, scarlet fever, typhoid, malaria and brucellosis.

The Head of NIAID's Medical Mycology Section pointed out that although mild or fatal systemic mycoses of man are not contagious, their causative agents are present in widespread areas of man's environment. The fungi responsible for systemic mycoses may grow and reproduce indefinitely in soil and humus as saprophytes, yet retain the ability to produce lethal disease in man. They possess characteristics which enable them under proper circumstances to turn immediately from a saprophytic existence to parasitism of animal tissues.

Their adaptability and prevalence, Dr. Emmons noted, indicates that medical mycology does not deal with "a bizarre group of rare fungi but with molds and yeasts with which man may be in daily contact." He also pointed out that the systemic mycoses remain difficult to treat while morbidity and mortality from bacterial disease have been reduced greatly by antibiotic therapy.

Causative agents referred to by Dr. Emmons included Coccidioides immitis, Nocardia asteroides, Histoplasma capsulatum, Cryptococcus neoformans, Aspergillus fumigatus, Absidia corymbifera and species of Rhizopus. Any of these can cause fatal mycoses and can be isolated easily and regularly from soil habitats. Other fungi which cause potentially fatal diseases are assumed on good evidence to have saprophytic existence and several fungi which cause chronic but rarely fatal diseases have been isolated from man's environment.

The NIAID mycologist discussed in detail several of the more familiar saprophytes which sometimes play a lethal role, including Crytococcus neoformans, the causative agent of cryptococcosis. C. neoformans is readily isolated in pigeon droppings on window ledges of office buildings and beneath roosting sites in open attics of old buildings in cities, as well as in barns and haymows on farms.

"These are virulent strains of the fungus, indistinguishable from those found in fatal cases of cryptococcal meningitis," Dr. Emmons said, stressing that "the public health implications of the common occurrence in man's environment of a fungus capable of causing fatal meningitis need careful evaluation."

SOME EXPERIMENTAL FUNGAL INFECTIONS ENHANCED BY CORTISONE

Experimental Candida infections in mice are enhanced to a far greater degree by cortisone than those due to Histoplasma capsulatum or Cryptococcus neoformans, according to a report appearing in the Journal of Clinical Investigation. Drs. Donald B. Louria, Nancy Fallon, and Harry G. Browne, authors of the report, received grant support from the National Institute of Allergy and Infectious Diseases.

Although many experimental infections are known to flourish following steroid administration, there is very little information on the relative disposition of the various species of fungi to disseminate in the presence of cortisone, nor is there any definitive data on the mechanisms involved or on tissue localization.

The investigators found that the administration of large amounts of cortisone produced a marked increase in the mortality of mice infected with Candida but does not enhance infections due to H. capsulatum or C. neoformans no matter what the quantity of inocula used.

Tissue census studies made by the scientists suggested that in cortisone-treated mice infected with this strain of C. albicans the increased mortality was related specifically to

increased multiplication of the fungus in the kidney. Although the exact mechanisms underlying renal susceptibility to infection are not yet delineated, detailed histological studies indicated that renal localization of infection in normal and cortisone-treated mice was related to rupture of the fungus into tubular lumina. Once within the tubule the fungus appeared to be protected from host defenses and multiplied uninhibitedly. Whereas extensive intra-tubular residence was uncommon in normal mice injected with large quantities of cells, it occurred uniformly in cortisone-treated animals.

On the basis of their investigations, Dr. Louria and his associates concluded that the time of cortisone administration appears to be highly significant in determining the degree to which Candida infection may be amplified. A single day's delay in treatment markedly reduces the effects of the hormone. When cortisone is administered 7 days after infection amplification of infection becomes negligible.

RICKETTSIAL DISEASES

Q FEVER INFECTION IN CATTLE FOUND TO OCCUR IN ALL PARTS OF THE UNITED STATES

Data now available demonstrate conclusively that Q fever occurs among dairy cattle in all parts of the United States. State or local health and agricultural groups in 26 States participated in recent surveys which confirm and expand earlier findings and prove that there is considerable bovine infection in 19 States not previously surveyed. These data are reported by Dr. Lauri Luoto in Public Health Reports. Dr. Luoto is on the staff of the Hamilton, Montana, Rocky Mountain Laboratory of the National Institute of Allergy and Infectious Diseases.

Over 24,000 herds were tested, including dairy cattle along the supposedly infection-free eastern seaboard, where high levels of infection were demonstrated. An unexpectedly high herd infection rate was encountered in most sections of the country, ranging from 1 to 65 percent. A significant spread of infection has been noted among herds in Idaho, Montana, and in other States. The increase of bovine Q fever, even within herds maintained under dispersed rural conditions, suggests its propensity for becoming a greater source of human infection.

Milk specimens and serums were tested for antibody against Coxiella burnetii, causative organism of Q fever, using the capillary-tube agglutination test (CAT). This procedure, used in Q fever studies since 1952, has been found to be specific, sensitive and reproducible. A close correlation

exists between the presence of the agent in milk and of agglutinating antibody in milk or serum of individual animals. The test works effectively under field conditions, detecting infection in herds and animals in areas where rates of infection among individual cows are only a fraction of 1 percent.

Not only were herd infections widespread, but a high percentage of infection occurred among animals within herds, ranging above 50 percent within some herds. Even in areas where infections are infrequent, high levels of infection exist within individual herds. For example, in Montana, where only 1 percent of herds are infected, up to 72 percent of cows in herds were positive.

In view of the widespread prevalence of bovine Q fever throughout the United States*, Dr. Luoto believes that information on associated human infections is urgently needed. Human infections occur and are diagnosed in areas where Q fever is known to exist in animal reservoirs. Human Q fever is already recognized as a public health problem in some areas. In Southern California at least 300 human cases were detected, and 350 cases were associated with sheep in Northern California during epidemiological studies in 1948-49.

The true incidence of human infection within this country is unknown because many cases may go unrecognized. Even during an acknowledged epidemic in Idaho during 1958, most of 93 laboratory-confirmed cases reported were diagnosed by about 10 percent of the local physicians, many of whom had diagnosed cases of Q fever in previous years. In many respects the failure to recognize this disease in man is similar to the situation existing when brucellosis in man was first associated with a disease of cattle. Based on present knowledge concerning Q fever, Dr. Luoto thinks it likely that human infections will become increasingly widespread and perhaps even now are occurring unrecognized.

Regardless of future implications, the presence of C. burnetii in animals and their products and environment should be of concern to agricultural, industrial and public health groups. Concerted nationwide studies of the occurrence and epidemiology of the disease are needed to help define the problem, particularly as it relates to the possibility of increasing incidence of human disease.

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*California, Wisconsin, Ohio, Iowa, Texas, Arizona, Idaho, Oregon, Washington, Montana, Wyoming, Utah, S. Dakota, Minnesota, Nebraska, Illinois, Michigan, Georgia, N. Carolina, Maryland, Pennsylvania, New Jersey, New York, Connecticut, Massachusetts, Hawaii. More limited data suggest occurrence in Louisiana, Mississippi, Virginia, Colorado, New Mexico, N. Dakota, Kansas, Missouri, Kentucky.

SPOTTED FEVER RICKETTSIAE STUDIED FOR IMMUNOLOGICAL CLUES TO AID DIAGNOSIS AND TREATMENT

Using a test based on neutralization by specific antiserums of toxins produced by various rickettsiae of the spotted fever group, Dr. E. John Bell and associates of the National Institute of Allergy and Infectious Diseases Rocky Mountain Laboratory have derived information of value in diagnosis and specific prophylaxis with vaccines. Their findings were published in the Journal of Immunology.

The toxins produced by R. rickettsii (Rocky Mountain spotted fever) and by R. conorii (boutonneuse fever) could be easily differentiated, and all strains in each species were found to be similar. It was shown that the organisms of Kenya typhus, Indian tick typhus, and South African tick-bite fever are closely related to, or identical with, that of boutonneuse fever. An organism isolated from the tick, Amblyomma americanum, was distinguishable from all other organisms under consideration, but has some reactions which show a relationship between it and R. conorii and the rickettsiae of Siberian tick typhus. The rickettsia of Siberian tick typhus, with the exception of the cross reaction noted above, was also distinguishable from other strains of the group.

These results further indicate the antigenic complexity of this group of rickettsiae and provide an elegant method for differentiating the members of this family of agents which has a world wide distribution.

FLUORESCENT ANTIBODY TECHNIQUE USED TO IDENTIFY TWO TICK-BORNE DISEASES

Working at the Rocky Mountain Laboratory of the National Institute of Allergy and Infectious Diseases, Drs. Willy Burgdorfer and David Lackman have used the fluorescent antibody technique in two studies at the Hamilton, Montana laboratories, one on Colorado tick fever virus, the other on Rocky Mountain spotted fever. Their report of the identification of the virus of CTF appears in the Journal of Bacteriology. The study concerned with visualization of R. rickettsii by fluorescence is reported in the Journal of Infectious Diseases.

By means of homologous antibody labelled with fluorescein-isothiocyanate, the investigators identified 34 CTF virus isolates in suckling mice with suspensions of Dermacentor andersoni, the northwestern species of wood tick which transmits CTF. They also used blood samples of three species of rodents collected in nature. Control tests performed simultaneously indicate that the reactions which occurred were specific.

The fluorescent antibody technique as a means of identifying isolates of CTF virus in suckling mice is of practical value in studies on the ecology of CTF. Using this method, it is now possible to make frequent isolations of virus either from the tick vector or from the blood of rodents that serve as hosts for the immature stage of ticks. In the past each isolate had to be identified by time-consuming neutralization tests in suckling mice.

Using the same technique in another investigation, Drs. Burgdorfer and Lackman have succeeded in identifying R. rickettsii, the etiological agent of Rocky Mountain spotted fever, in smears of gut tissues from experimentally infected D. andersoni. In smears of infected nymphal ticks the organisms stained consistently with a very bright fluorescence. In those prepared from adult ticks several weeks after molting, the rickettsiae fluoresced less strongly, although they were still detectable. Experiments are in progress to determine if this phenomenon is connected with the effect of molting on the virulence of the organism, reported previously by another investigator.

(The investigators learned, upon completion of this research, that the fluorescent antibody technique has been used at the PHS Communicable Disease Center on rickettsiae of Rocky Mountain spotted fever, using individual ticks.)

In this study, all of the staining reactions, from strong fluorescence to a notably decreased amount, were specific as shown by the results of control experiments. Inhibition could be observed regularly with unlabelled immune serum, and staining was not detected when heterologous conjugate was applied or when smears of normal ticks were treated with specific conjugate. In addition, triturates of ticks found positive by the technique under study always produced clinical or serological evidence of Rocky Mountain spotted fever infection in guinea pigs. Triturates free of rickettsiae did not cause the disease when injected into guinea pigs.

HIGHLIGHTS OF PROGRESS

IN RESEARCH ON

ARTHRITIS AND METABOLIC DISEASES

1960

Items of Interest on Program Developments and Research Studies

Conducted and Supported by the National Institute of Arthritis

and Metabolic Diseases

The scientific advances described on the following pages are categorized under various disease headings, but this has been done mainly for the convenience of the reader and not because of any real separation between the fields as far as fundamental knowledge is concerned. As research progress continues, it becomes increasingly apparent that underlying all the fields is a substructure of basic research knowledge common to all the medical sciences. Obtaining this basic knowledge is one of the primary activities of the National Institute of Arthritis and Metabolic Diseases.

Many times the results of basic studies have no direct application to treatment, such as the major finding made this year about the manner in which hormones directly affect key metabolic processes within a cell. Although this discovery has no immediate clinical use its implications are obvious since hormonal activity is involved in many diseases. Occasionally, however, basic studies lead directly to dramatic developments. Basic laboratory studies on burn shock at the Institute have now culminated in an effective gamma globulin treatment for severe post-burn infections that are otherwise fatal.

In addition to its original areas of interest--rheumatic diseases, metabolic disorders, gastroenterology, hematology and nutrition--the Institute has recently acquired three new fields of responsibility, pediatrics, orthopedic surgery and dermatology. The diversity of these fields makes it even more necessary to strengthen basic research. Today, Institute scientists and grantees are focusing their attention on the root causes of disease. Mere palliation is no longer enough, for no matter how effective symptomatic relief becomes it cannot prevent disease. It is this prevention, not treatment, that is the ultimate goal of medical research.

RHEUMATIC DISEASES

During the past ten years, under the auspices of the Institute, a tremendous increase has occurred in research on arthritis and the other rheumatic diseases, and great improvements have been made in the treatment of these disorders. Since cortisone was first used in 1948, the pharmaceutical industry has developed a whole spectrum of corticosteroid drugs, many of which have been tested at the Institute, for the relief of pain and inflammation. Thousands of arthritics have benefitted from these successors to cortisone, but unfortunately the drugs cannot cure the underlying disease. Thus, research must continue to be directed toward basic causes. Recent studies have added further support to the concept that several of the rheumatic diseases result from a state of hypersensitivity, and may be diseases of autosensitivity in which a person becomes overly sensitive to substances that are normally present within his body.

STUDY SHOWS THAT ARTHRITIS IS MORE WIDESPREAD THAN BELIEVED

Evidence that rheumatoid arthritis may be at least six times more widespread than has been generally assumed was reported to the annual meeting of the American Rheumatism Association by grantees of the National Institute of Arthritis and Metabolic Diseases. A survey of employed workers at the Oak Ridge (Tenn.) National Laboratory indicates that many cases of rheumatoid arthritis may go undiagnosed because the disease runs a benign, remittent course with mild-to-moderate symptoms and little disability.

In the past, the best available estimates put the number of arthritis sufferers at about two and a half percent of the total population of the United States. The present study of 331 craftsmen and their foremen shows that mild rheumatoid arthritis affects about 15 percent of regularly employed plant workers. Usually, the course of the disease was mild to moderate and disability was rare.

The study was begun after it was found that one out of four employees came to the Oak Ridge National Laboratory's dispensaries because of a rheumatic complaint. The survey was conducted over a 28-month period and included medical screenings of the workers which were done monthly for the first 16 months and quarterly thereafter. A carefully trained nurse was instructed to ask pertinent questions and to make brief joint examinations, and men with swollen joints were immediately referred to the plant physician for examination. Uniform diagnostic criteria, established by the American Rheumatism Association, were used throughout the study, and included morning stiffness, pain on motion or tenderness, observed swelling, swelling of two or more joints and symmetrical swelling.

The NIAMD grantees, Drs. Thomas A. Lincoln, Medical Director, Oak Ridge National Laboratory, and Sydney Cobb, University of Pittsburgh, reported that by the end of the study five percent of the screened employees had been diagnosed for "definite" rheumatoid arthritis and an additional ten percent for "probable" rheumatoid arthritis--making a total of 15 percent who at some time during the 28-month study were classified for "probable" or "definite" rheumatoid arthritis.

Increased frequency of rheumatoid arthritis with age has also been observed. Of 93 men between 50 and 66 years of age, ten percent had "definite" and an additional 12 percent had "probable" rheumatoid arthritis.

The investigators pointed out that the final percentage of men affected by rheumatoid arthritis would probably have been higher if medical observations had extended beyond the 28-month period. This is also borne out by the fact that during the study's first month only two percent met the criteria for probable or definite rheumatoid arthritis. As observations continued, increasing numbers of employees were admitted to these two diagnostic criteria. It was apparent that a prolonged period of observation was necessary to properly classify these patients since most of them were free of joint swelling most of the time.

Standard latex agglutination tests were also done on sera from the patients. Though the tests were negative in most of these cases of mild arthritis, the patients had significantly more latex agglutinating substance in their sera than do normal individuals, but had less than clinical cases of rheumatoid arthritis.

It thus appears that rheumatoid arthritis may be a disease that is much more common (and benign) than has been supposed, the NIAMD-supported investigators reported, and they concluded from their study that rheumatoid arthritis affects a substantial portion of a group of regularly employed men. In contrast to the more severe cases usually seen in clinics and consulting rooms, these cases seem to run a benign remittent course with mild to moderate symptoms and little disability.

DISCOVER NEW SIDE EFFECT OF CORTICOSTEROID DRUGS

Clinical investigators at the National Institute of Arthritis and Metabolic Diseases in collaboration with the National Institute of Neurological Diseases and Blindness have discovered a heretofore unnoticed

side effect of corticosteroid therapy--the production of slight opacities in the lens of the eye. These opacities (posterior subcapsular cataracts) in most cases cause no impairment of vision and do not require surgery. They are distinct from the more serious "senile" type of cataracts which result in failing vision and require surgical removal of the lens.

The lens opacities were first noticed in four rheumatoid arthritis patients at the Institute who were receiving prolonged therapy with synthetic corticosteroids. The eye lesions consisted of small filament-like spots present on the posterior surface of the lens and occurred in both eyes. Such cataracts are generally associated with exposure to toxic agents, eye disease or injury, or exposure to radiation, but since none of these factors were implicated in the four cases, the NIAMD and NINDB investigators began a careful examination of 66 rheumatoid arthritis patients available for study at the Institute. Of this group, 44 had received steroid therapy while 19, who served as the control group, had not.

The investigators, Drs. Roger L. Black, Richard B. Oglesby, Ludwig von Sallman and Joseph J. Bunim, found the posterior subcapsular cataracts in 17 of the 44 steroid-treated patients (39 percent) and none in the non-steroid treated control group. The appearance of these opacities was uniform and easily distinguished from the more common cataracts. In 11 of the 17 cases the opacities were visible with an ophthalmoscope. Only six of these 17 patients had experienced any symptoms from the opacities and in only four of these six was there any measurable loss of vision. The visual loss was slight (a change from 20/20 to 20/40 vision), and no case required surgery.

There was a striking relationship between the amount of corticosteroid received and the occurrence of the lens' opacities. For purposes of the study the steroid-treated patients were divided into three groups: a "low dosage" group who had received on the average less than 10 mg. daily of prednisone or its equivalent; a "midrange dosage" group receiving 10 to 15 mg. daily; and a "high dosage" group receiving 16 mg. or more per day. No patients in the low dosage group had developed opacities whereas 23 percent of the midrange dosage group and 75 percent of the high dosage group had developed the eye lesions. The duration of the corticosteroid therapy was also significant in the production

of the opacities but to a lesser extent than the dosage level.

The relatively young age of the patients developing the opacities (10 of the 17 were less than 50 years of age) further supports the evidence that the posterior subcapsular cataracts were not of the senile type. Additionally, of all the patients over 50 in the study, none of the non-steroid-treated ones were found to have posterior subcapsular cataracts whereas half of the steroid-treated patients in this age group developed the condition.

No specific corticosteroid preparations were implicated; there was no apparent difference between the various drugs used (cortisone, hydrocortisone, prednisone, triamcinalone, 6-methyl prednisolone and dexamethasone) as far as their likelihood of producing the opacities. The mechanisms by which the corticosteroids produced the lesions are still purely speculative. One interesting and still unexplainable finding was that males seemed to be more susceptible than females; the opacities were found in 12 males but only five females.

The NIAMD and NINDB investigators, who reported the study to the annual meeting of the American Rheumatism Association, emphasized that the opacities do not constitute a major hazard to vision and should not be alarming. To date, no patient has developed serious visual defects. It would therefore seem that in most cases this eye complication by itself would not indicate the need for discontinuing steroids.

VALUE OF GOLD INJECTIONS IN RHEUMATOID ARTHRITIS CONFIRMED

The distinct value of gold salts in the treatment of arthritis has finally been clearly demonstrated in a British study reported by a recent Visiting Scientist at the National Institute of Arthritis and Metabolic Diseases. Injections of gold salts were first used in the treatment of rheumatoid arthritis in 1927 and since then have been widely employed by many physicians with varying degrees of success, but few well controlled studies have been made of this form of treatment. Such studies are not easy to perform since the disease is an extremely variable one and it is very difficult to obtain two similar groups of patients, and experimental group and a control one. This has now been accomplished in the British study and has provided conclusive proof of the value of gold therapy in rheumatoid arthritis.

The double-blind study was carried out in 24 treatment centers in Great Britain under the sponsorship of the Research Sub-Committee of the Empire Rheumatism Council. Dr. Eric G. L. Bywaters, who reported the study to The American Rheumatism Association, was one of the participants and is Professor of Rheumatology at the University of London. He returned to London after a brief stay as a Visiting Scientist at the National Institute of Arthritis and Metabolic Diseases.

Two hundred out-patients were involved and were divided equally into two groups, comparable in age, sex and duration of illness. Only patients who had had rheumatoid arthritis for more than one year and less than five were included in the study, and none had been previously treated with gold salts. The "gold" group was given weekly injections of 50 milligrams of metallic gold (as sodium aurothiomalate) over a period of twenty weeks, while the control group received weekly injections of similar appearance but containing virtually no gold (1/100,000 the quantity given the gold group). All received simple supportive measures such as rest, splints, aspirin and physical therapy.

Improvement in the gold group became noticeably greater than in the control group after the first three months and was measured in a variety of ways: (1) functional capacity--more patients became and remained able to carry on normal work and physical recreation; (2) joint involvement--fewer joints became newly affected or reactivated with the disease, while more joints that had been affected became quiescent and remained that way; (3) blood tests--hemoglobin levels and red cell sedimentation rates improved by the sixth month in the gold group and the sheep cell agglutination test became less positive; (4) number of analgesic tablets taken--at the start both groups were taking an equal number of aspirin tablets or equivalent per day. A reduction in aspirin dosage which was considered significant by the investigators was seen in the gold group at the sixth month from the start of the treatment and continued to the 18th month.

X-rays of both groups of patients were also taken at intervals during the study but these did not show any material difference in the two groups.

One of the biggest problems with gold therapy has always been the production of side effects, and these occurred to a significant degree in this study also. Toxic effects, largely dermatitis, were three times more common and were more severe in the gold-treated than in the control group. Complications

were noted in about 25 percent of the gold group and 12 percent of the control group. Although the skin reactions were troublesome in a significant number of the cases, the NIAMD Visiting Scientist reported, various new drugs such as British Anti-Lewisite (BAL--a heavy metal binding compound) and adrenal cortical steroids can reduce the severity of such side effects and lessen the dangers of this form of therapy.

"A later assessment will be made after a further one year's follow-up, but to date the advantage clearly lies with the gold-treated group," Dr. Bywaters concluded.

PRESENT TREATMENT FOR SLE FOUND VALUABLE

Systemic lupus erythematosus, the so-called Red Wolf disease (Lupus-wolf), has been the subject of a large scale study by a grantee of the National Institute of Arthritis and Metabolic Diseases. Systemic lupus erythematosus (SLE) is a pernicious, multisystem disease entity with certain similarities to rheumatoid arthritis; it is difficult to diagnose because there is no classic disease pattern. Any system may initially be affected and heal; months or years later the same or another area may become involved.

A ten-year, 400-patient study made by Dr. Edmund L. Dubois of the University of Southern California School of Medicine showed that SLE was almost as common as pernicious anemia, leukemia, acute rheumatic fever or serum sickness at the 3500-bed Los Angeles County General Hospital. One case of SLE was seen for every 14 of rheumatoid arthritis, and 88 percent of the patients were female.

The investigator found that the most effective therapy for milder attacks was bed rest, salicylates and antimalarial drugs. If this treatment failed or if the patients were critically ill or had neurological or renal involvement, corticosteroid treatment was employed. A segment of the study comparing 50 patients treated with dexamethasone with comparable series of patients treated with older adrenocortical steroids showed that the newer steroid, while 20 times more powerful than hydrocortisone and 8 times more powerful than methylprednisolone, brought patients little additional relief. Because of increased side-effects, namely edema, hirsuitism, and peptic ulceration, dexamethasone was not preferred.

Although the ultimate cure of systemic lupus erythematosus must await further knowledge of its basic cause, the NIAMD-

supported study showed that presently available forms of therapy (salicylates, antimalarial compounds, and corticosteroids) can effectively inhibit for a significant time the grave changes that occur in the disease. The study was reported and was the subject of an editorial in The Journal of the American Medical Association.

EXCESS BODY WEIGHT HAS LITTLE EFFECT ON OSTEOARTHRITIS

Scientists at the National Institute of Arthritis and Metabolic Diseases (NIAMD) have found that excess body weight bears little if any relationship to the cause of osteoarthritis in mice. The research was reported by Dr. Leon Sokoloff of the Laboratory of Pathology and Histochemistry, and associates, to the annual meeting of the American Rheumatism Association, and has shown that osteoarthritis has a much more complex cause than simply that of extra weight-bearing by the joints of the body.

Osteoarthritis is a "wearing away" disease of the joints which is much more common than rheumatoid arthritis but, as a rule, less damaging. The disease seems to result from a combination of aging, damage and normal wear and tear and affects older persons most frequently. Although it has long been supposed that obesity aggravates osteoarthritis in man--presumably by burdening the joints with additional weight--there is little direct evidence of this, and so the present study was done to determine more clearly the relationship between obesity and osteoarthritis.

The NIAMD scientists performed several groups of experiments. In the first one, mice which did not normally become obese were fattened on a diet containing 60 percent vegetable shortening. The mice became 75 percent fatter than litter mates fed a normal diet, but pathological studies of their hind limbs done after 16 months showed that, for the most part, even this severe a degree of obesity did not harm the joints. The same fattening procedure was also used on rats, which are generally less susceptible to joint disease. In these experiments the obese animals did show slightly more arthritis than normally-fed litter mates, but their joint disease was very mild.

The most conclusive studies were done with mice belonging to the STR/1N strain. This strain is naturally fat and, in addition, has a high incidence of osteoarthritis. All STR/1N mice develop osteoarthritis by about one year of age. To determine whether the animals' weight was directly related to

their joint disease, the scientists restricted their weight gain by feeding them a low calorie diet. It was found that the weight restriction had no effect on either the occurrence of osteoarthritis or its severity.

Further proof of the lack of association between obesity and osteoarthritis came from studies of hybrid mice which had resulted from the mating of STR/1N mice (fat, arthritis-susceptible mice) with mice from a thin, arthritis-resistant strain. The hybrids were practically as fat as their arthritic fathers but had almost as little arthritis as their thin mothers, thus confirming the dissociation between weight and arthritis.

"Under the conditions of these experiments there was no consistent effect of obesity on the development of degenerative joint disease," Dr. Sokoloff reported. Although the obesity-producing diet did slightly influence the development of joint disease in the rats, the results of the other experiments make it unlikely that the effect was simply the mechanical consequence of excessive weight borne by the joint. Possibly, certain metabolic alterations are involved, although no single metabolic abnormality has yet been found.

The NIAMD scientists concluded that the cause of osteoarthritis is a much more subtle and complex biological problem than simple mechanical stress.

IMPORTANT DIFFERENCES FOUND IN JOINT DISEASES

Evidence that, contrary to traditional belief, the joint diseases found accompanying ulcerative colitis are basically different from rheumatoid arthritis has been reported by Dr. Currier McEwen and a biostatistician associate, Miss Claire Lingg, both of New York University Medical College. The study supported by a grant from the National Institute of Arthritis and Metabolic Diseases, was reported to the Sixth Interim Scientific Session of the American Rheumatism Association. It involved 52 patients and indicated that there are significant differences both in clinical symptoms and in response to blood tests for rheumatoid factor, between persons with rheumatoid arthritis and patients with joint disorders found associated with colitis.

Estimates indicate that about seven percent of patients suffering from ulcerative colitis also have swollen and inflamed joints, until now thought to be due to rheumatoid disease. In the group of 52 patients studied, 33 had arthritis of peripheral joints, a much higher incidence than was expected.

The Institute-supported investigators found that arthritis accompanying colitis differed from rheumatoid arthritis in several respects: serological reaction for rheumatoid factor was consistently absent; subcutaneous nodules did not appear; affected joints were not of symmetrical distribution; the arthritis was evanescent and not chronic or destructive and therefore did not result in joint destruction or deformity; and prevalence among males and females was about equal whereas in rheumatoid arthritis, three times as many women are affected as men. In a great majority of cases, the appearance of colitis antedated the appearance of arthritis. In a few cases, both diseases started simultaneously or the arthritis preceded the colitis. Flareups of ulcerative colitis frequently coincided with flareups of arthritis.

Fourteen of the 52 patients under study were shown to have spondylitis (arthritis of the spine) which resembled the classical type of ankylosing spondylitis that occurs independent of ulcerative colitis.

STUDY OF SJOGREN'S SYNDROME SUPPORTS AUTOIMMUNITY CONCEPT

A serological study of 21 patients with Sjogren's Syndrome, a disorder often accompanied by arthritis, has provided additional support for the concept of autoimmunity as a possible cause of disease. The Study, which may throw some light on the basic nature of rheumatoid arthritis, was reported by Dr. Joseph J. Bunim, Clinical Director of the National Institute of Arthritis and Metabolic Diseases, as part of the annual Stoneburner Lecture Series at the Medical College of Virginia.

The concept of autoimmunity has become popular within recent years to explain the occurrence of certain diseases whose etiology is still unknown. According to this concept, it is possible for an individual to become sensitive to substances normally present in body cells and tissues, and to produce antibodies against these substances. The resultant antigen-antibody reactions in the tissues are thought to lead to the pathological changes noted in the disease. This concept has been useful in explanations of Hashimoto's disease of the thyroid gland. Individuals with this disorder were found to have circulating antibodies in their serum which reacted with their own thyroglobulin. Presumably, the antibodies were reacting with the thyroglobulin in the thyroid gland and causing the tissue damage characteristic of the disease.

As a result of this work, many studies were begun by various investigators to re-examine other diseases of unknown etiology, in an attempt to discover if they might also be expressions of autoimmunity. At the present time rheumatoid arthritis is being considered as such a disease by many investigators, partly because the "rheumatoid factor" which is present in the serum of rheumatoid arthritics has many of the properties of an antibody. In a related disease, systemic lupus erythematosus, it has been found that the serum contains a whole family of antibodies, some against normal cell nuclei, some against cytoplasm, and others against sub-components of both nuclei and cytoplasm.

With these findings in mind, Dr. Bunim and his associates at the National Institute of Arthritis and Metabolic Diseases made a comprehensive serological study of patients with Sjogren's Syndrome to determine what antibodies might be present in their serum. This syndrome is characterized by hypofunctioning of both the tear and salivary glands and is often accompanied by rheumatoid arthritis. Any two components of the ocular-salivary-arthritic triad are sufficient to establish the diagnosis. Aside from the occurrence of the arthritis, the syndrome is of particular interest because the pathological changes found in both the tear and salivary glands are strikingly similar to those seen in the thyroid glands of persons with Hashimoto's disease.

The NIAMD serological study showed that an abnormally active immunological response was present in patients with Sjogren's Syndrome. Almost all the patients gave positive responses to the bentonite flocculation test and sensitized sheep cell agglutination test for rheumatoid arthritis, even though only 8 patients out of 21 had definite clinical signs of the disease. The majority of patients were also found to have a serum constituent that had a specific affinity for cell nuclei, and in this respect they resembled patients with systemic lupus erythematosus. Another interesting finding was the presence of an antibody to human thyroglobulin (14 percent of the cases) which was similar to the one found in Hashimoto's disease.

Dr. Bunim reported that these findings demonstrate that Sjogren's Syndrome patients exhibit much greater immunological reactivity than those with rheumatoid arthritis alone, and the diversity of the humoral antibodies may indicate a basic derangement of antibody production.

This NIAMD research thus provides further support for the developing concept of autoimmunity, and indicates that immune mechanisms may be playing an important part in the pathogenesis of these diseases.

DIABETES AND OTHER METABOLIC DISORDERS

The field of diabetes offers many problems for research, for although insulin and diet will control most cases, there are still large gaps in our knowledge of this common metabolic disease. Unsolved questions relate to its basic cause, the many biochemical and physiological changes that take place in body fluids and cells, and the influence of heredity. Of particular interest at the present is the mode of action of the new oral hypoglycemic drugs which have replaced insulin injections in thousands of diabetics. Though these new compounds are widely used, they are not effective in all cases of diabetes, and there is increasing evidence that individual patients may become resistant to one or more of them as time goes on. Undoubtedly, the new drugs have provided a convenient form of treatment but they are not the final answer.

NEW FINDINGS ON ORAL ANTIDIABETIC DRUGS REPORTED

A review of the current status of oral antidiabetic drugs was presented by scientists from the National Institute of Arthritis and Metabolic Diseases, grantees of the Institute, and other investigators at the annual meeting of the American Diabetes Association. Highlights of the meeting included indications that secondary failures of the drugs occur in up to 35 percent of patients, and evidence that prolonged treatment with tolbutamide (in animals) does not exhaust the beta cells of the pancreas as some researchers have feared.

Tolbutamide, the first of the oral antidiabetic drugs, became available in 1957 and since then two others, chlorpropamide and phenformin, have been made available to physicians. At the present time it is estimated that more than one-third of all known diabetics in the United States are now receiving this oral therapy instead of regular insulin injections. Even though these drugs are widely used, the way

in which they accomplish their blood-sugar-lowering action is still not fully understood, nor is it known whether the drugs, in the long term, will represent a significant improvement in the treatment of diabetes.

A continuous evaluation of 355 patients treated with the oral drugs over a four year period by one group of researchers reporting at the meeting showed that primary failures amounted to approximately 30 percent, and that of the remaining 70 percent of patients, up to 35 percent became secondary failures. They felt that this percentage of secondary failures might eventually be even higher if patient groups are followed for a longer period of time. Some investigators believed that the secondary failures may possibly result from a compensatory increase in the production of insulinase, an hepatic enzyme that degrades insulin in the body.

Other investigators reported that their overall results with phenformin in 107 difficult diabetic patients were not as good as the results which have been reported by physicians who used it in all diabetics. Although the drug was found useful either alone or in combination with insulin, side effects considerably limited it and were noted in half of the patients taking doses over 150 mg.

In another reported clinical study the effects of tolbutamide on the levels of serum lipoproteins were investigated, since it was thought that any increases in the beta lipoproteins may parallel the progression of the vascular degeneration seen in diabetes. The study revealed that there was an increase, although a relatively small one, in the beta lipoproteins in patients who have been converted from insulin to the oral drug and who remained "well controlled" as far as their blood sugar levels were concerned. This raises the question whether the patient who is considered "stabilized" with respect to blood glucose levels is really controlled with respect to his fat metabolism.

In the past, considerable evidence has indicated that tolbutamide facilitates the release of insulin from the pancreas. Therefore it has been a matter of concern that this form of "stimulation," if it occurs chronically or at all, might eventually exhaust the pancreatic beta cells when their number or function is already reduced in the diabetic patient.

Studies of prolonged tolbutamide administration in dogs were reported at the meeting which now indicate that the beta cells do not become exhausted. The animals had three-fourths or more of their pancreas surgically removed in order to diminish their beta cell reserve without producing diabetes, and received repeated intravenous glucose tolerance tests over a period of 13 to 17 months. None of these operated animals became diabetic, and as a group they showed no trend toward an over-all decrease in tolerance as compared to control animals. Tolbutamide did not seem to affect the degree of pancreatic regeneration. A comparison of the estimated amount of remaining pancreas at operation with its appearance and weight at autopsy showed an increase of 1.5 to almost fourfold in both experimental and control groups.

In experiments of a somewhat similar nature, other investigators reported that in rats made "subdiabetic" by alloxan treatment, prolonged tolbutamide administration did not cause permanent deterioration. In fact, some of these alloxanized animals insular function improved.

INSULIN ANTAGONISTS FOUND IN UNTREATED DIABETES

The presence of substances antagonistic to insulin in the plasma of patients with diabetes mellitus who have never received insulin therapy has been shown in recent studies by Dr. James B. Field, Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases. These studies shed provocative light on an important possible cause of diabetes other than pancreatic inability to produce insulin.

The likelihood that many cases of diabetes are due to substances in the patient's body which interfere with provision of an effective insulin concentration to peripheral tissues has been considered by endocrinologists for many years. A cause of diabetes of this sort is under investigation because numerous diabetic patients require more than forty units of insulin a day, the level needed by a patient whose pancreas has been removed in the treatment of some other disease and whose diabetes is thus due to his inability to produce his own insulin.

Inhibitors or antagonists to the action of insulin having the properties of antibodies have previously been found by Dr. Field and by other investigators in the plasma of patients with chronic insulin resistance requiring amounts of insulin in excess of two-hundred units per day. Temporary insulin resistance, accompanied by the presence of increased amounts of inhibitors, has also been found in diabetic acidosis or coma and during infection. In general there has been no good

correlation between the titer of insulin antibody and a patient's daily insulin dosage.

The recent studies of Dr. Field differ from previous observations in that insulin antagonist was detected in the plasma of two of four new diabetic patients prior to insulin therapy, indicating the presence of a substance interfering with the action of insulin which is not an antibody elaborated in response to administered insulin. Clinically, there were no distinguishing features between the two cases with antagonist and those without. They all had high blood sugars, large amounts of sugar and ketone bodies in the urine without severe acidosis and required insulin for control of their diabetes in approximately equal amounts. Inhibitor could no longer be detected once the diabetes was controlled.

Detection of this apparently new type of insulin antagonist in the NIAMD studies provides a lead for further investigation of a variety of factors affecting insulin production, binding, transport and action at the peripheral tissue level. The possibility exists that the antagonist is a hormone of pituitary origin, perhaps related to growth hormone. The studies, and a review of factors affecting insulin action, were reported by Dr. Field in The Journal of the American Diabetes Association.

MENTHOL FOUND TO HAVE HORMONE-LIKE ACTIVITY

Menthol, the substance that gives peppermint its characteristic odor, has been found to have an effect similar to that of the female sex hormone progesterone on the metabolism of galactose. This discovery, by scientists at the National Institute of Arthritis and Metabolic Diseases, indicates that menthol may be another rare example of a non-steroidal compound that produces some of the same physiological effects as a hormone.

Menthol's hormone-like effect became apparent during studies of galactosemia, a relatively rare hereditary disorder of children. Infants with this disease are unable to tolerate galactose, a sugar present in milk, since they lack one of the enzymes necessary for galactose metabolism. If milk is allowed to remain in their diet, it quickly creates a toxic condition leading to mental retardation, cataract formation, liver damage and early death.

The basic nature of the disease was discovered in 1955 by scientists at the National Institute of Arthritis and Metabolic Diseases who first made the biochemical diagnosis and pinpointed the exact enzyme that was deficient. Further studies of the disease led to the discovery that injections of the hormone progesterone enabled galactosemic children to metabolize at least some galactose. Apparently the hormone enables the body to overcome the enzymatic block. This finding was reported last year and set an important precedent for research on other metabolic disorders caused by enzyme deficiencies.

The hormone-like activity of menthol was discovered by accident during detailed in vitro studies of progesterone's effect on galactose metabolism. Menthol was being employed to study some of the reactions when it was noted that the compound had the same ability to stimulate galactose metabolism as progesterone. Oral doses of menthol were then tried in two galactosemic children and enabled them to metabolize the same amount of galactose as they would have with progesterone injections. These findings were reported at the Federation meetings by Drs. T. D. Elder, S. Segal, E. S. Maxwell and Y. J. Topper of NIAMD.

The therapeutic implications of the findings have yet to be explored since only tracer doses of galactose have been used in the clinical trials. Also, the mechanism by which either progesterone or menthol exert their effects in vivo is not yet known. Menthol may prove to be of particular value in further NIAMD studies of this phenomenon since the menthol molecule is a much smaller and simpler one than that of progesterone.

NEW BLOOD TEST DEVELOPED FOR GALACTOSEMIC CHILDREN

A new blood test for monitoring how completely milk and milk-containing foods have been eliminated from the prescribed diet of children with galactosemia has been developed by Drs. Henry Kirkman and Elizabeth Maxwell, of the National Institute of Arthritis and Metabolic Diseases. (Dr. Kirkman is now at the University of Oklahoma Medical School.) The test measures the amount of galactose-1-phosphate present in red blood cells, and since this substance is thought to be the most important toxic agent in the disease the test will be valuable in managing galactosemic patients.

The exact biochemical defect in the disease was first demonstrated by Dr. Herman Kalckar and associates at NIAMD in 1956, who found that galactosemic individuals lacked a single enzyme necessary to completely metabolize galactose. Because of this inherited enzyme deficiency, galactose-1-phosphate accumulates in the blood and tissues and produces the characteristic symptoms.

Although diagnosis now presents little difficulty (Dr. Kalckar's work provided a specific biochemical test for the disease) keeping galactosemic children under strict dietary control is a continuing problem. It is difficult to find processed foods which contain no milk or milk products and the clandestine ways children have of getting food often result in unsuspected breaks in their prescribed diet.

The new test now provides a way to determine how much galactose, if any, the child is getting and is sensitive enough to indicate a break in the diet as short as one day. It can be performed in several hours with equipment usually found in a medium-sized hospital or Public Health center and uses the same reagents as the diagnostic test developed by Dr. Kalckar. These reagents are presently available commercially from the Sigma Biochemical Supply Company in the form of a diagnostic kit.

The NIAMD scientists reported on the development of the new test in the Journal of Laboratory and Clinical Medicine.

SPECIAL DIET PREVENTS MENTAL EFFECTS OF PHENYLKETONURIA

Convincing evidence that a special diet can prevent or delay severe mental deficiency in children with phenylketonuria (PKU) has now been provided by a grantee of the National Institute of Arthritis and Metabolic Diseases. His study, involving every case of PKU reported up to December, 1959, shows that a child's IQ permanently drops five points for every ten weeks after birth that the diet treatment is delayed.

Phenylketonuria is an "inborn error of metabolism" whose most disastrous consequence is severe mental retardation. The disorder is caused by the lack of a single enzyme in the body, and an enzyme needed to convert phenylalanine, one of the amino acids, into tyrosine, another amino acid. Because the enzyme is lacking, this metabolic "step" is blocked and abnormal amounts of phenylalanine accumulate in the blood.

Previous studies by other investigators have shown that although there is no cure for the basic defect, a diet low in phenylalanine prevented to some extent the mental retardation of PKU. Until now, however, the degree of effectiveness of the diet on the mental deficiencies had been uncertain because of small sizes of diet-treated patient series and varying degrees of mental retardation normally found in the disease.

Dr. W. Eugene Knox, an NIAMD grantee at Harvard Medical School, has pooled and compared the results of all reported treated cases of PKU and has assessed the value of the treatment by comparing results of the treated patients of different ages with the natural history of the disease in untreated patients. He found that in a group of 43 patients treated before age three, there were 18 times as many with IQ's above 60, twice as many with normal electroencephalograms, and fewer with seizures than in a group of 20 that had received no dietary therapy. Those treated before 16 months, compared with those treated between 16 months and three years of age, had four times as many with IQ's above 60 and fewer seizures. The early treated group also had more normal electroencephalograms. Forty-three patients with PKU over three years of age were treated without impressive changes in their mental and neurological state.

For the whole group, the NIAMD supported investigation showed that the final IQ was negatively correlated with age at the start of treatment in a highly significant manner. Over the three-year period studied, a minimal loss of nearly five points in IQ occurred each ten weeks that treatment was delayed. This finding points to the obvious importance of diagnosis of PKU as soon as possible after birth. To this end a relatively simple diagnostic test was developed last year by Dr. Bert LaDu of NIAMD in Bethesda; the test can be performed in ten minutes on finger-prick samples of blood.

This study "provides the final evidence that phenylalanine in some way causes these central nervous system abnormalities. The primary enzymatic defect leading to accumulation of phenylalanine is therefore linked through a causal chain to the mental deficiency. This means in practice that treatment to reduce accumulation of phenylalanine in patients with phenylketonuria should not be denied," Dr. Knox reported. The work was reported in Pediatrics.

RAPID SCREENING TEST FOR WILSON'S DISEASE DEVELOPED A spot test for detecting children who may develop Wilson's disease later in life has been developed by grantees of the National Institute of Arthritis and Metabolic Diseases. The test is simple and rapid and measures the amount of the plasma copper protein, ceruloplasmin, in a blood sample. Its routine use as a screening procedure may make it practicable to prevent or delay the onset of the symptoms and signs of Wilson's disease.

Wilson's disease is believed to result from abnormal copper metabolism and is characterized by an hereditary deficiency of ceruloplasmin and progressively fatal degeneration of the liver. Excessive amounts of copper accumulate in the liver, brain and urine, and the urinary excretion of amino acids also increases. It is not yet clear what the relation is between these metabolic changes, but it appears that the accumulated copper is responsible for the pathologic changes seen in the disease.

The NIAMD grantees believe that the chief clinical value of the new test will lie in screening apparently healthy children to detect those who are deficient in ceruloplasmin. This is important, since the signs and symptoms of the disease rarely occur before the age of seven or eight years, although the deficiency in ceruloplasmin appears to be present from birth. Therefore, if latent Wilson's disease, or hypoceruloplasminemia, is demonstrated, measures may be instituted to minimize the absorption of copper from the diet, and to promote the excretion of copper already absorbed.

The basis of the test is that ceruloplasmin catalyzes the oxidation of the polyamine, paraphenylenediamine (ppd). The test is made on a sample drop of either plasma or serum which is placed on a paper strip treated with ppd dihydrocholoride. The intensity of the blue spot which then appears under the test sample is compared with that made by a sample of normal serum or plasma; it is possible to determine whether the sample contains more or less than about 20 mg. of ceruloplasmin per 100 ml. of plasma or serum. The normal value is 18 to 35 mg. per 100 ml. (Prepared paper strips, capillary tubes, and standard serum for clinical testing are now available from the Ortho Research Foundation, Raritan, New Jersey.)

Ceruloplasmin levels are naturally low in newborn infants but they appear to be consistently above 18 mg. per 100 ml. by the second month of life. Therefore, it seems that the spot test can be used as a screening procedure in infants at any time after the age of three months, perhaps most conveniently at a time when routine immunizations are given, the NIAMD grantees reported.

"Since Wilson's disease is an uncommon condition and practically the only cause of persistent deficiency of ceruloplasmin, usually in the range of 0 to 15 mg. per 100 ml. of serum, confirmatory studies, including quantitative analysis for ceruloplasmin, can be made in the relatively small number of children in whom positive results of the spot tests are obtained. In children who show persistent ceruloplasmin deficiency, Wilson's disease is reasonably certain to develop, but early diagnosis may make it possible to prevent or delay the appearance of hepatolenticular degeneration," the investigators concluded.

The development of the spot test was reported in The American Journal of Medicine by Drs. Philip Aisen and Julian B. Schorr, Anatol G. Morrell, Ruth Z. Gold and Dr. I. Herbert Scheinberg, Albert Einstein College of Medicine and Bronx Municipal Hospital Center, and the Columbia University School of Public Health and Administrative Medicine, New York, New York.

DIET, NOT HEREDITY, MAY CAUSE HEMOCHROMATOSIS

Hemochromatosis and hemosiderosis, disorders in which abnormal amounts of iron are deposited in body tissues, have been thought to be examples of inborn errors of metabolism. A comprehensive study of 211 cases of these disorders by grantees of the National Institute of Arthritis and Metabolic Diseases (NIAMD) has now indicated that alcoholism and nutritional factors, rather than genetic ones, are etiologically implicated.

Hemochromatosis, or Bronze diabetes, is a rare disease that is usually ascribed to an inborn error of metabolism. It is characterized by widespread deposits of hemosiderin (an iron-protein complex that is probably a storage form of iron) and presumably results from exaggerated absorption of iron from the intestinal tract. The pigment deposits cause degenerative and fibrotic changes in the involved organs, particularly the liver and pancreas, and lead to the classic triad of bronzed skin, diabetes and cirrhosis of the liver. Hemosiderosis, a

closely related disorder, is distinguished from hemochromatosis by the lack of tissue damage in the organs even though pigment deposits are present.

The present NIAMD-supported study of 211 autopsied cases of hemochromatosis and hemosiderosis was undertaken at Boston City Hospital and reported by Drs. Richard A. MacDonald and G. Kenneth Mallory, of the hospital, Harvard Medical School and Boston University School of Medicine, in the A. M. A. Archives of Internal Medicine.

The autopsied cases of these diseases occurred in 25,295 post mortems between the years 1917 and 1956 and were separated into three groups according to pathological criteria; there were 57 cases of hemochromatosis, 104 cases with a slight amount of hemosiderin in various organs, classified simple hemosiderosis, and an intermediate group of 50 cases, classified advanced hemosiderosis. From careful analyses of the autopsy diagnoses, clinical records, and all available microscopic sections the investigators pieced together a disease pattern which has led them to suspect that alcoholism and nutritional factors, rather than genetic ones, are of prime etiological importance.

Originally, one of the bases on which hemochromatosis was considered an inborn error was the reported appearance of the disease in two or more members of the same family. In the present series of over 200 cases, however, there was only one patient known to have a sibling with hemochromatosis.

Of greater significance, the NIAMD grantees believe, was their finding that hemochromatosis and hemosiderosis frequently occurred together with fatty nutritional (alcoholic) cirrhosis, and that 85 percent of patients with hemochromatosis whose clinical records were available used alcohol to excess. Three-fourths of those who denied alcohol had a history of a grossly inadequate diet. Among the hemochromatosis patients 45.6 percent had either fat in the liver or cirrhosis with fat, in addition to pigment cirrhosis; among the advanced hemosiderosis patients 86.2 percent of the cirrhotic ones had elements of fatty nutritional cirrhosis as did 28.5 percent of non-cirrhotic ones; and among the simple hemosiderosis patients 69.0 percent of the cirrhotic ones had fat in the liver as did 32.5 percent of the non-cirrhotic ones.

No definitive differences between the three groups were found when clinical and pathological features were compared, suggesting that the three groups were not sharply distinct

entities but were variants of a single condition. It was also noteworthy that transfusions were not a significant factor in the study. In most patients excess iron in the tissues appeared to have been the result of absorption from the diet.

The histologic picture of pigment cirrhosis was often consistent with that resulting from a preexisting fatty nutritional cirrhosis in which fat had been mobilized from the liver, the NIAMD grantees reported, and they believe that the "idiopathic" type of hemochromatosis may be a variant of nutritional cirrhosis, in which the same factors that contribute to the cirrhotic process lead to iron absorption. Other cases of hemochromatosis may be due to a combination of factors, such as anemia or hematologic disease causing excessive iron absorption, with transfusion or orally administered iron further contributing to the amount of iron in the tissues. In addition, they concluded, there may coexist factors that contribute to, or in themselves lead to cirrhosis.

ETHER ANESTHESIA FOUND TO CAUSE METABOLIC CHANGES

Grantees of the National Institute of Arthritis and Metabolic Diseases have found that ether anesthesia produces marked changes in normal carbohydrate metabolism. Their research indicates that ether inhibits specific metabolic pathways and suggests that it may be more advantageous to feed surgical patients with intravenous fructose rather than glucose since fructose metabolism is less affected than is the metabolism of glucose.

It has been known for many years that ether anesthesia affects carbohydrate metabolism, increasing the blood levels of glucose, pyruvic acid and lactic acid. Numerous investigators have also noted increases in serum inorganic phosphorus during ether anesthesia. What has not been clear, however, is whether the anesthetic causes a generalized depression of enzymatic processes or inhibits only specific metabolic pathways.

In an attempt to clarify ether's effect on carbohydrate metabolism NIAMD grantees have studied the metabolism of two sugars, fructose and glucose, in anesthetized subjects. The subjects were all volunteer patients without metabolic disorders, and since there was no surgery performed during the anesthesia the metabolic changes could be attributed to ether

alone. Both glucose and fructose tolerance tests were performed under anesthesia, and the results were compared with those obtained in the same patients on the days preceeding and following the administration of ether.

As expected, the levels of glucose, pyruvic acid and lactic acid were all increased during anesthesia. However, in contrast to the metabolic disturbance found after surgery, most of the changes noted did not persist; they returned to the control range within 24 hours. After surgery, these changes are known to persist for at least 48 hours. The NIAMD grantees also found that during anesthesia intravenous infusions of glucose could not be assimilated as rapidly as they normally had been. Infusions of fructose, on the other hand, could be assimilated just as rapidly under anesthesia as they were under normal conditions. Since the metabolism of glucose involves different pathways from those of fructose, the investigators interpret the results as indicating that ether inhibits only certain metabolic pathways without causing a generalized interference with biochemical processes.

The scientists supported by NIAMD believe that these metabolic alterations found during anesthesia suggest several improved means for managing patients during surgery. Preanesthetic administration of carbohydrate may be indicated since a high content of hepatic glycogen is known to protect the liver from damage during anesthesia. Also, the use of fructose may have the advantage of being more protective than glucose since studies by other investigators have shown that in both normal and diabetic patients the liver removes intravenously administered fructose twice as rapidly as it does glucose in comparable situations, and fructose tolerance was relatively unimpaired in this anesthesia investigation.

"In addition, the results of the present study indicate intravenous fructose is assimilated more rapidly than intravenous glucose by subjects during ether anesthesia. Consideration of these findings may be important for the optimal alimentation of surgical patients if the present trend continues toward longer operations and more operations in the geriatric age group," the investigators concluded.

The study was reported by Drs. W. R. Drucker, Christine Costley, Robert Stults, William D. Holden, James Craig, Max Miller, Nancy Hofmann and Hiram Woodward, of Western Reserve University, Highland View Hospital and the University Hospitals of Cleveland, Cleveland, Ohio, in the Journal Metabolism.

PITUITARY RESERVE FUNCTION MEASURED BY NEW TEST

A new method to determine the "reserve capacity" of the pituitary gland for secreting ACTH (adrenocorticotropin) has been developed by grantees of the National Institute of Arthritis and Metabolic Diseases. The new test indicates how well the pituitary is able to respond to above-normal demands for ACTH, as in stress, injury or infection, and may help clarify the role played by this master gland in various endocrinological disorders. The development of the test was a logical continuation of research done by Dr. Roy Hertz of the National Cancer Institute on amphenone, a compound which inhibits the production of cortisol by the adrenal gland.

The ideal way to study pituitary secretion of ACTH would be to measure the levels of the hormone itself in the blood, but since available methods are not sufficiently sensitive to measure circulating ACTH (under most physiologic conditions) an indirect type of measurement must be used. This has been made possible in the new test by employing an agent known as SU-4885* (an analogue of amphenone) to inhibit the synthesis of cortisol (hydrocortisone) by the adrenal gland. The falling level of cortisol then stimulates the pituitary to "compensate" by producing more ACTH and the additional ACTH, in turn, causes the adrenal gland to secrete large quantities of 11-desoxycorticosteroids which can be readily measured in biologic fluids as 17-hydroxycorticoids.

Thus, in normal subjects, SU-4885 induces a rise in total blood and urinary corticoids. Since this response to SU-4885 is dependent upon a rise in ACTH secretion, the compound affords (in patients with no frank adrenal insufficiency) a sensitive means of testing the reserve capacity of the pituitary gland to secrete ACTH. To explore the possible diagnostic use of the phenomenon, the NIAMD grantees studied the effects of SU-4885 in 71 patients whose endocrinological status had been clearly defined by conventional methods.

In certain endocrine states the results obtained were as anticipated but in others revealing information was derived as to the state of pituitary-adrenal integrity. For example, in Addison's disease (adrenal insufficiency) there was no appreciable increase in urinary 17-hydroxycorticoids; in hypopituitarism no increase in the already subnormal excretion of urinary 17-hydroxycorticoids was noted; in Cushing's disease all patients with active adrenal hyperplasia showed responses which were greater than the average response of

normal subjects; some patients with a chromophobe adenoma of the pituitary had normal responses and some did not, indicating that in this condition there is frequently, but not invariably, damage to the ACTH-secreting mechanism so that varying degrees of hypoadrenocorticism result; in acromegaly some of these patients had limited capacities to secrete ACTH under test conditions; all patients with cachexia exhibited "limited pituitary reserve," indicating that severe malnutrition may frequently be associated with impairment of the pituitary capacity to respond to the demands of a disease or stress situation.

The results obtained in a variety of clinical situations appear to substantiate the validity of the SU-4885 test, and the NIAMD grantees concluded that it now provides a means of testing the reserve capacity of the pituitary-adrenal system to satisfy extraordinary physiologic needs for cortisol. The findings were published in The Journal of Clinical Endocrinology and Metabolism by Drs. G. W. Liddle (formerly of the staff of the National Heart Institute), H. L. Estep, J. W. Kendall, W. C. Williams and A. W. Townes of Vanderbilt University School of Medicine, Nashville, Tennessee.

* 2-methyl-1,2-bis(3-pyridyl)-1-propanone

GERMFREE ANIMALS USED TO STUDY NUTRITION

The use of germfree animals at the National Institue of Arthritis and Metabolic Diseases (NIAMD) has helped to clarify the role played by man's intestinal bacteria in supplying essential nutrients.

At the recent Fifth International Congress on Nutrition held in Washington, D. C., Dr. Floyd S. Daft and Ernest G. McDaniel reported on their studies of the growth-promoting effects of penicillin and ascorbic acid in rats, and the production of folic acid by gastrointestinal bacteria.

Earlier studies showed that both penicillin and ascorbic acid could prevent a deficiency in pantothenic acid, an essential B vitamin, in conventional rats, but the exact mode of action of these compounds had not been clear. In the reported studies, germfree rats were fed a pantothenic-free diet containing either 200 mg. percent penicillin or ascorbic acid at a level of two percent in the diet. Neither of these compounds prevented the B vitamin deficiency in the germfree animals, indicating that the previously observed growth-promoting effects of these compounds are mediated

through their biochemical action on intestinal bacteria.

The NIAMD investigators also clarified aspects of folic acid deficiencies. It is well known that conventional rats do not require a dietary source of folic acid. However, symptoms of folic acid deficiency can be produced in rats on a folic-free diet, if sulfa drugs are administered. This suggests that folic acid is ordinarily made available to the host animal by bacteria in its gastrointestinal tract and that this essential vitamin is no longer synthesized during suppression of the intestinal flora by sulfonamides.

The germfree studies confirmed this hypothesis by showing that germfree rats develop severe folic deficiency after about 50 days on a folic-free diet. Normal growth stops, severe anemia develops and the animals die if not treated. These results indicate that the rat does have a requirement of folic acid which, under normal circumstances, is satisfied through synthesis of this essential nutrient by the intestinal bacteria. Such studies furnish significant analogies for the elucidation of the role of intestinal microflora in the nutrition of man.

"NIBBLING" HAS SPECIFIC EFFECTS ON REGULATION OF METABOLISM

The laboratory rat is a nibbler while man is an eater of full meals, and therein lies a difference that can have a marked effect upon experimental studies of obesity, atherosclerosis and diabetes. This has been reported by grantees of the National Institute of Arthritis and Metabolic Diseases who found that the difference in the rate of ingestion between nibbling and eating spaced, full meals affects the regulation of metabolism of fat, carbohydrate and protein as well as the synthesis or release of several protein hormones. As a result of their findings, the grantees believe that experimental animals such as the rat should be trained to eat full meals as men do if they are to be used in studies of human disease.

It has long been assumed that as long as food enters the gastrointestinal tract, no matter how it gets there, an animal would handle it in the same way. The NIAMD-supported studies, however, comparing body composition and metabolic activities of force-fed animals with those of animals eating ad libitum, show that great differences in intermediary metabolism are produced by the manner of eating. In rats, meal eating as contrasted with nibbling resulted in (1) a 50 to 100 percent increase in the body fat, (2) a 10 to 20 percent decrease in protein synthesis, (3) a three-fold increase in glucose-6-phosphate dehydrogenase activity, (4) a decreased I^{131} uptake by rat thyroid and diminished thyrodial I^{131} decay, (5) development of diabetes in partially pancreatectomized rats, and (6) enhanced atherogenesis in monkeys and chickens.

These findings, the grantees believe, indicate that full meal eating is associated with a more economical use of calories, leading to obesity and that meal eating differentially affects the traffic over alternate metabolic pathways. Also, the rate of thyroid stimulating hormone (TSH) release by the pituitary may be decreased by meal eating.

"It appears unimportant at this time to determine whether man is basically a converted nibbler, but it does appear that extremely significant information would be obtained if it were found that man reacts to meal eating as contrasted to nibbling as does the rat. If man does react in this manner, it is quite possible that a number of diseases associated with abnormalities in fat, protein, and carbohydrate metabolism may be caused or aggravated by eating habits.....it appears to us that animal experimentation that is designed to explore diseases and metabolic abnormalities which afflict man should use the feeding patterns of man," the investigators concluded.

The work was reported by Drs. Clarence Cohn and Dorothy Joseph of the Department of Biochemistry, Medical Research Institute, Michael Reese Hospital, Chicago, Illinois, in Metabolism.

GASTROENTEROLOGY

Peptic ulcer, ulcerative colitis, ileitis and other diseases of the digestive system afflict thousands of Americans, in many cases causing death or prolonged and expensive hospitalization. Their importance is reflected in the fact that diseases of the digestive system rank second only to heart disease in the number of illnesses requiring attendance by a physician. These diseases are only beginning to get the research attention they deserve since too few investigators had been interested in them in the past. The momentum is increasing, however, due to the availability of new techniques such as radioisotope studies, improved methods of determining gastric secretions, and the use of specialized motion picture equipment to photograph the activities of internal organs during health and disease.

RESEARCH REVEALS DIRECT CAUSE OF ULCER PAIN

Studies of more than five hundred ulcer patients by grantees of the National Institute of Arthritis and Metabolic Diseases have shown that, contrary to common belief, ulcer pain is not caused by the direct chemical irritation of tissue by hydrochloric acid but results from a disturbance in the motor activity of the stomach and duodenum.

The investigators used a variety of techniques to clarify the mechanism of ulcer pain. One was the "acid test," the instillation of hydrochloric acid into the stomachs of patients with active ulcers who had experienced pain within the previous 24 to 48 hours, or who were having spontaneous pain at the time of the study. Contrary to the 85-90 percent of "positive" results reported by other investigators, the NIAMD-supported group found positive results in only 36 percent of the patients, that is, only about a third of them developed typical ulcer pain or experienced continuation or accentuation of spontaneous pain.

Because of this discrepancy, the investigators were led to make fluoroscopic studies of the motor activity of the stomach and duodenum in ulcer patients during periods of pain. Using a new fluorocinematographic device developed especially for this research, the grantees were able to make X-ray motion pictures of gastrointestinal motor activity over a prolonged period of time. Concurrently, they recorded intraluminal pressures in various parts of the stomach and duodenum and correlated the pressure changes with gastric movements.

They found that although duodenal ulcer pain was accompanied by a significant increase in motility, at the same time there was a significant delay in gastric evacuation. The coordination of motor activity that serves to "pump" out material from the stomach was temporarily lost, probably because of increased resistance at the pyloric sphincter. In contrast, no alterations in motor activity were found in ulcer patients who were not having pain. In most instances pain relief occurred spontaneously when evacuation activity was resumed. This may account for the fact that pain often disappears after food is ingested and resting motor activity becomes converted to evacuation motor activity. (Similarly, the relief of pain following vagotomy and cholinergic blocking agents may be related to their inhibitory effect upon gastrointestinal motor activity.)

The authors feel that the mucosal engorgement accompanying the ulcer may lower the threshold to hydrochloric acid, permitting the acid and perhaps other stimuli to initiate the reflex disturbance in motor activity which is the direct cause of the pain.

These NIAMD-supported gastroenterological studies are continuing. This particular phase was reported in the Annals of Internal Medicine by Drs. E. C. Texter, Jr., G. R. Vantrappen, H. P. Lazar, E. J. Puletti and C. J. Barborka, of the Veterans Administration Research Hospital, Passavant Memorial Hospital, and Northwestern University Medical Center, Chicago, Illinois.

CLOSING MECHANISM BETWEEN STOMACH AND ESOPHAGUS IDENTIFIED

The long-disputed mechanism by which food in the stomach is prevented from returning through the esophagus during digestion has been demonstrated independently by two groups of NIAMD-supported investigators. Their studies indicate that an "intrinsic sphincter mechanism" exists in the lower portion of the esophagus, in the area where it is attached to the stomach. A circular bundle of esophageal muscle fibers apparently closes off the esophagus by contracting, and although this mechanism is not anatomically distinct, the area has definite characteristics which are different from the remainder of the esophagus. Determination of its exact location is of considerable value to the surgical management of patients with disorders of the esophagogastric junction.

Using a device for simultaneously taking fluoroscopic motion pictures of the esophagus while measuring its intraluminal pressure, NIAMD grantees at Northwestern University Medical Center have demonstrated that approximately the distal inch of the esophagus acts as

a physiological sphincter (a functional closing mechanism). A high pressure zone occurs in this segment of the esophagus and apparently results from the continuous contractions of the circularly arranged muscle fibers. The pressures in this segment are such that during each phase of respiration the whole zone or part of it acts as a barrier against reflux.

Through surgical studies on experimental animals, NIAMD grantees at the University of Virginia have now established the "intrinsic" nature of the mechanism. The sphincteric action is in the esophageal segment itself and is not dependent upon such extrinsic mechanical factors as the movements of the diaphragm. When the terminal segment of the esophagus was resected or surgically isolated there was a loss of reflux control; control returned when the isolated segment was restored to its normal position. The surgical researchers have also devised an operation which offers promise as a reconstructive measure in patients who have lost function in the lower portion of the esophagus or had it removed. The surgeons produce an artificial extrinsic sphincter by wrapping a flap of the diaphragm around the esophagus where it joins the stomach. Respiratory motions then cause the flap to close off the stomach.

The Northwestern NIAMD grantees, Drs. G. Vantrappen, E. Clinton Texter, Jr., Clifford J. Barborka and J. Vandenbroucke reported on the work in the American Journal of Medicine, and the work of the Virginia investigators was reported by Drs. Phyllis Ingram, James Respess and William Muller, Jr., in Surgical Forum.

SEVERITY OF LIVER DISEASE INDICATED BY NEW TEST

A new ammonium tolerance test which can aid in evaluating liver disease has been developed by an Institute grantee. The new test is simple and safe and consists of administering a provocative oral dose of ammonium citrate and then measuring the resultant levels of blood ammonia. It provides a sensitive index of the existence of portal-systemic shunts and a method of estimating the severity of parenchymal liver disease.

Studies of the metabolism of ammonia in man during the past few years have made it clear that elevations of ammonia concentration in the peripheral blood are dependent almost invariably upon

either severe degrees of hepatic parenchymal disease or due to the existence of spontaneously developed or surgically constructed shunts around the liver.

The present study of the effects of the diagnostic value of an oral dose of ammonium citrate was conducted in patients with various known degrees of liver damage. Some of the patients in each group had demonstrable shunts in the form of esophageal varices. Five to ten grams of ammonium citrate were administered to the patients and their peripheral venous blood ammonia concentration was then measured at intervals of 30, 60, 120, and 240 minutes. Toxic manifestations other than occasional mild cerebral symptoms were not observed.

The NIAMD-supported investigator, Dr. William V. McDermott, Jr., of Harvard University, reported in Surgical Forum that in patients with severe liver disease the induced hyperammoniemia persisted for many hours, probably because of defective urea synthesis. It was not possible solely on the basis of the rate of disappearance of blood ammonia, however, to determine consistently the presence or absence of varices in these patients.

On the other hand, patients with normal or well compensated liver function showed no appreciable rise in blood ammonia levels unless a portal shunt existed. If esophageal varices were present, then there was a rapid rise in blood ammonia levels within 30-60 minutes, followed by a rapid fall over the next two to three hours. "In several instances this method of defining the presence or absence of esophageal varices has proved to be more accurate than radiographic techniques," the NIAMD grantee concluded.

ALCOHOL'S EFFECT ON LIVER CLARIFIED

Studies of alcohol's effect on liver tissue have shown that alcohol (ethanol) stimulates the synthesis of fatty acid in the liver. This finding, confirmed by both in vitro and in vivo studies, helps explain the production of a fatty liver in chronic alcoholism.

Although fatty degeneration of the liver has long been known to be associated with chronic alcoholism, the mechanism by which alcohol produces this effect has not been clear. Now, both in vitro and in vivo animal studies by grantees of the National Institute of Arthritis and Metabolic Diseases have contributed substantially to the understanding of this action. The studies have shown that ethanol in divided and moderate doses causes the concentration of fatty acid in the liver to increase by indirectly

supplying DPNH, an enzymatic co-factor involved in fatty acid synthesis. The finding was reported by Drs. C. S. Lieber, L. M. DeCarli and Rudi Schmid, Boston, Massachusetts to the recent meeting of the American Society for Clinical Investigation at Atlantic City, New Jersey.

The investigators injected tracer doses of acetate-1-C^{14} into male rats and then, after feeding the animals either glucose or ethanol, measured the amount of acetate-1-C^{14} incorporated into hepatic fatty acids. The ethanol-treated rats, in comparison to their glucose-fed littermates, showed not only an increase in the concentration of total hepatic fatty acids but also a 40 percent increase in the incorporation of C^{14}. Further *in vivo* studies confirmed this stimulatory effect on fatty acid synthesis in the liver.

To uncover the mechanism of this stimulation, *in vitro* studies were done on liver slices incubated in ethanol, and demonstrated a three-to-eight fold increase in the incorporation of acetate-1-C^{14} into fatty acids. In contrast, ethanol had no effect on fatty acid synthesis in adipose tissue.

The NIAMD-supported scientists found that ethanol's effect is due to an increased formation of DPNH which occurs in the liver when ethanol is oxidized. (DPNH in its oxidized form, DPN, is a co-factor required by many enzymes and is intimately involved in fatty acid synthesis.) Thus, as the ethanol is oxidized, the amount of available DPNH increases and this in turn leads to an increase in fatty acid synthesis. These findings, both in the intact animal and in liver slices, now provide an explanation for the common finding of fatty livers in patients with chronic alcoholism.

An alternate explanation for the stimulating effect of ethanol on hepatic fat synthesis has also been proposed elsewhere by Dr. B. B. Brodie and colleagues of NHI. According to this view, large single doses of alcohol interfere with the normal endocrine (pituitary) control of fat transport and result in the mobilization of excessive quantities of fatty acids from the adipose tissues of the rat. These free fatty acids circulate to the liver where they are re-combined with glycerol and deposited in the parenchyma as triglycerides.

BASIC RESEARCH

There is a continuing requirement for basic research by the Institute, for despite the gratifying progress that is being made

in controlling some of the chronic diseases there is still far too little known about their fundamental cause and nature. Much of this needed knowledge can only come from basic research; it is the common thread that runs through the fabric of all the medical sciences. Many of the studies now being pursued at the Institute have no immediate application to the treatment of a particular disease, but they are vitally necessary to provide basic facts about living systems. Of increasing interest are Institute studies in the fields of epidemiology and biometrics, which are concerned with the reasons why some population groups are more or less susceptible to disease than others. A thorough analysis of these reasons--nutritional, climatic, genetic, etc.--promises to provide scientists with a better understanding of many diseases.

MAJOR FINDING ON HORMONE ACTION REPORTED

Studies by Drs. K. Lemone Yielding of the Institute's Arthritis and Rheumatism Branch, and Gordon M. Tomkins, Laboratory of Biochemistry and Metabolism, have shown that certain female sex hormones can alter the molecular structure of a key catalyst in mammalian cell metabolism, by fragmenting it into four smaller, catalytically altered molecules. This is the first time that a hormone has been found to affect directly the physical structure of an enzyme; previous studies by other investigators have suggested that hormones exert their biochemical influences by participating chemically in metabolic reactions, undergoing an oxidative or reductive change.

As indicated by the NIAMD studies, the reversible physical inactivation of a strategically placed cell enzyme determines the direction which the cell's metabolic processes will take--toward energy-yielding chemical breakdown, or toward cell-building protein synthesis. This finding of molecular alteration is the first concrete illumination of a basic regulatory mechanism which determines protein synthesis and, indirectly, reflects on associated cell growth or cell proliferation.

The studies reported describe the effect of four steroid hormones on crystalline glutamic dehydrogenase (GDH), an important enzyme which facilitates the energy-yielding chemical degradation of glutamic acid. The absence or inactivation of this enzyme tips the scales toward the diversion of glutamic acid to protein synthesis by the cell.

In <u>in vitro</u> experiments Drs. Yielding and Tomkins found that diethylstilbesterol, estradiol, and progesterone, all belonging to the group of female sex hormones, inhibited the normal GDH reaction. Further investigation demonstrated that the hormones

promoted dissociation of the large GDH enzyme molecule, which has a molecular weight of 1,000,000, into four subunits of molecular weight of 250,000. These smaller molecules were enzymatically inactive with respect to GDH activity. Inactivation of the enzyme was reversed in the presence of adenosine diphosphate, a normal cell metabolite. Cortisone, another steroid hormone tested, did not affect the molecular architecture of GDH and correspondingly did not interfere with chemical degradation of glutamic acid. This selective inhibition by female sex hormones was demonstrated with GDH from beef, chicken, and rat liver, and rat testis, kidney and heart.

A report on these studies by Drs. Yielding and Tomkins appeared in the Proceedings of the National Academy of Sciences.

NEW STUDIES HELP EXPLAIN HOW GENES CONTROL CELL

Today, one of the most fundamental problems in biology is the manner in which genes determine and control biochemical processes in cells. Basic studies of this control mechanism, which suggest that genes are turned on and off and may explain how this is done, have been reported by scientists at the National Institute of Arthritis and Metabolic Diseases.

Within the past few years, support has been gathered for the hypothesis that genes determine the metabolic pattern in an organism by determining the structure of enzymes, that is, by ordering the sequence of the amino acid building blocks in enzymes. This hypothesis does not account for the fact that some cells of an organism produce enzymes which other cells do not, even though all the cells have the same chromosomes and presumably the same genes. One possible explanation of this is that a mechanism exists for turning genes "off" or "on" and allowing differentiation among the cells.

Dr. Bruce Ames and Miss B. Garry, of the Laboratory of Biochemistry and Metabolism, have approached this problem of genetic control through a study of six of the thousands of enzymes in bacterial cells. These specific enzymes are synthesized by the cell in order to produce histidine, an essential amino acid, and each enzyme catalyzes one step of a multi-step process, or metabolic pathway, the end product of which is histidine.

The NIAMD studies have shown that in Salmonella bacteria a feedback mechanism regulates the synthesis of the series of enzymes of histidine biosynthesis; the rate of enzyme synthesis slows down as the histidine pool increases. The

significant aspect of the finding is that the rates of synthesis of each of the enzymes in the series of enzymes involved in this particular metabolic pathway were repressed to the same extent by histidine. (Other investigators have found that the rate of a single enzyme's synthesis will vary with the size of the pool of the end product, an occurrence called enzyme repression, but this is the first demonstration that a group of enzymes may respond in unison.) The phenomenon has been named "coordinate repression" by the Institute scientists and occurs regardless of the amount or even of the presence of the individual enzyme substrates available in the cell. The NIAMD scientists reported their findings in part in the Proceedings of the National Academy of Sciences recently, and additional aspects of the work will appear in the Journal of General Microbiology (in press).

This new observation is of interest in view of the genetic analysis of various histidine mutant strains of Salmonella bacteria. Dr. P. Hartman of Johns Hopkins University has isolated a series of different mutants of Salmonella that all require histidine for growth. Each mutant is damaged in one of the genes involved in making an enzyme required for histidine biosynthesis. These genes have been mapped on the Salmonella chromosome by Hartman and shown to be in a cluster. The investigations of the NIAMD scientists have shown that each type of mutant Hartman isolated is missing a different enzyme of the biosynthetic pathway. The genes could be associated with the enzymes they control. Hartman's genetic map in conjunction with the NIAMD biochemical studies has revealed the startling fact that the linear order of the genes on the chromosome corresponds to the sequence of the enzymes they control in the biosynthetic pathway.

In view of these two sets of observations, an attractive hypothesis, though one for which direct proof is lacking, is that perhaps the feedback mechanism for regulating histidine production operates at the gene level, and that in some way histidine can functionally "turn off" that length or section of the chromosome containing the genes of the histidine enzymes. Therefore, when the synthesis of the necessary enzymes stops, the production of histidine would stop. The controlling factor may be histidine itself, or a histidine-nucleic acid repressor which has a specific affinity for the histidine section of the chromosome.

Although Salmonella exhibits this orderly clustering of genes, it is not found in Neurospora or in yeast. The histidine genes in these organisms are scattered on the chromosome, and the investigators are studying them in more detail.

The basic NIAMD research involved in these studies provides a fuller understanding of how genetic information controls biological activity, regulating normal biochemical processes in health and, where enzymatic defects or omissions occur, apparently contributing to or causing biochemical derangements in "molecular" or metabolic diseases.

ABNORMAL HEMOGLOBINS SHOW EFFECTS OF GENETIC MUTATIONS

Institute scientists are studying the physical-chemical structure of abnormal hemoglobins to learn more about the effects of genetic mutations on protein synthesis. The studies have shown that the synthesis of the hemoglobin molecule is not controlled by a single gene, but rather that two "halves" of a molecule are synthesized separately--under the control of separate genes--and then joined together to form the complete molecule.

Within recent years it has become apparent that various metabolic disorders in man are associated with the inherited absence or decrease of a particular enzymatic activity. Phenylketonuria, galactosemia and alcaptonuria are all examples of hereditary disorders in which specific enzymes are either lacking or deficient. One possible mechanism for this apparent inhibition of activity is that a mutant gene has caused the production of a structurally abnormal enzyme. This concept of structural abnormality, however, is difficult to test directly, since it is rarely possible to obtain enzymes, especially human enzymes, in adequate purity and quantity for chemical and physical characterization.

One approach to this problem is to study other human proteins, even though they are not classified as enzymes, since the concepts concerning genetic control of protein synthesis apply to these other proteins as well as to enzymes. This is the approach that is being utilized by Dr. Harvey Itano and Elizabeth Robinson, of the Laboratory of Pathology and Histochemistry, in studies of the various types of human hemoglobin. These proteins (the hemoglobins) can be obtained in large quantity and in numerous genetically abnormal forms--hemoglobin S, C, etc.--and provide an extremely useful system for studying protein synthesis and structure.

The NIAMD study has shown that when hemoglobin molecules are placed in an acid medium they will dissociate into two unlike subunits. The molecules are composed of two pairs of amino acid chains, designated alpha chains and beta chains, and in an acid medium the molecules will split asymmetrically, that is, into

two alpha chains and two beta chains. When the medium is neutralized, the two unlike "halves" of the molecule recombine to form the complete molecule.

(Normal hemoglobin has two identical alpha chains and two identical beta chains. This is also true of the abnormal hemoglobins, but in them there is an alteration in the amino acid sequence, either of both alpha chains or of both beta chains. As yet no hemoglobins have been found that have alterations in their alpha chains and their beta chains, except in a rare situation in which a person has inherited both a hemoglobin abnormal in the alpha chain and a hemoglobin abnormal in the beta chain.)

The NIAMD scientists have found that all the various types of hemoglobin will undergo dissociation into half molecules and will then recombine not only with their own complementary halves but also with the complementary halves of other types. This phenomenon thus makes it possible to determine where the alteration exists in a hemoglobin of unknown structure. The "unknown" hemoglobin is mixed with one whose structure is known, and by analyzing the resultant combinations it is possible to determine in what chains, alpha or beta, the unknown is defective. This technique of "asymmetric recombination" has now been used to study many of the abnormal hemoglobins and has clarified the nature of their defects. Recently, the investigators reported in Nature that the phenomenon will take place in an alkaline medium as well as an acid one.

One of the most significant aspects of the NIAMD research is its demonstration that the synthesis of the entire hemoglobin molecule is not controlled by a single gene. Evidently, a separate gene controls the synthesis of each pair of chains, and after the two pairs are synthesized separately they join together to form the complete protein molecule. The hemoglobin studies are thus providing important information on protein synthesis and on the effect of mutations on protein structure.

TASTE TEST INDICATES INHERITED TRAITS

A taste test is being used by Dr. Baruch S. Blumberg, of the Institutes Program on Geographic Medicine and Genetics, to study one of the many inherited biochemical variations--polymorphisms--that exist among humans. The test employs the compound phenylthiocarbamide (PTC) which certain individuals can taste and others cannot. Since the tasting trait is believed to be genetically determined, the test provides a way to determine the presence or absence of a single gene in the population.

Many such polymorphisms exist in both animals and man, such as unusual types of human hemoglobin, certain synthesized metabolites in the body, and variations that are found among serum hemoglobin binding proteins taken from different individuals. Dr. Blumberg's interest in these biochemical variations stem from the fact that although they are apparently harmless in themselves, they may be affecting an individual's susceptibility to various diseases.

The PTC polymorphism was first reported in 1932, shortly after the compound was synthesized by a Dupont chemist, when family studies of the phenomenon indicated that the trait was a genetically determined one, tasters being dominant for the gene and non-tasters being recessive for it. Since then the ratio of tasters to non-tasters has been determined in many populations by various investigators. The most recent study was made by Dr. Blumberg in an Alaskan Eskimo population and helps to clarify the worldwide distribution of the tasting trait. The study was made in collaboration with Dr. A. C. Allison of the National Institute of Medical Research, London, and was reported by Drs. Allison and Blumberg in the journal Human Biology.

For many years after its discovery the PTC polymorphism was thought to be only another biochemical oddity until it was discovered that certain other compounds could also be tasted by PTC tasters but not by non-tasters. The interesting observation was made that in the chemical structure of all of these compounds there was a characteristic thiourea grouping of nitrogen, carbon and sulphur which accounted for the tasting phenomenon. This same thiourea grouping is present in a large series of anti-thyroid substances and known goitrogens, and several studies by other investigators indicate that non-tasters may be more susceptible to certain kinds of goitre than are the tasters. The possibility exists that there is some relationship between the PTC tasting polymorphism and the occurrence of thyroid disease and is being further explored.

In the Eskimo study, Dr. Blumberg found that the frequency of non-tasters was relatively high, 26 percent. This frequency is the same as that found in Western Europeans and comparable to the one existing in the white American population. It is in sharp contrast, however, to the very low frequencies found in American Indians, and the difference is in keeping with many other genetically controlled ones between American Indians and Eskimos which make it improbable that the two populations are related racially.

Further studies of these polymorphisms by the NIAMD scientist may provide important information about the genetic variations that exist among people and populations, and how these variations may influence health and disease.

STUDY SUGGESTS HAPTOGLOBINS ARE RELATED TO DISEASE

A geographic study of inherited serum proteins called haptoglobins suggests that there may be a relationship between these genetic characteristics and human disease. The study is headed by Dr. B. S. Blumberg of the Program on Geographic Medicine and Genetics, National Institute of Arthritis and Metabolic Diseases, NIAMD, and Dr. A. C. Allison of the British National Institute for Medical Research.

The haptoglobins are a family of serum proteins which bind hemoglobin. Although the presence in the blood of three major inherited forms of these proteins has been known for several years, Drs. Blumberg and Allison have now found four additional phenotypes including one in which no haptoglobins are detectable.

In any one individual the pattern of serum haptoglobins is controlled by two genes, termed haptoglobin ^{1} and haptoglobin^{2}, which are not sex linked and are fully expressed in the heterozygote. (Homozygous individuals possess only one type of haptoglobin gene; heterozygous individuals possess both types and they have a more varied pattern of serum haptoglobin).

The investigators have "mapped" the distribution of haptoglobins and the frequency of the haptoglobin genes in various populations throughout the world. They have found that the haptoglobin^{1} gene tends to be more common in tropical regions in North and South America and Africa, but not in Asia. Dr. Blumberg and Allison believe that the distribution of these various forms of haptoglobins in different human populations may bear some relationship to disease susceptibility. Studies on the haptoglobins suggest that they may be important in the nutrition of some populations since the absence of haptoglobins results in a loss of hemoglobin in the urine. In regions where iron deficiency is endemic this loss could be a contributing factor in anemia. The scientists have also confirmed previous findings that the concentration of haptoglobins rises in different diseases--in rheumatoid arthritis, for example, and a number of other acute and chronic inflammatory diseases.

The investigators are now attempting to isolate the different haptoglobin types and to break down their components biochemically in an effort to determine the differences and similarities among types. Many aspects of this study have been reported in the scientific literature. One of the more recent publications appeared in The Annals of Human Genetics.

BLOOD TRANSFUSIONS MADE SAFER FOR NEWBORN INFANTS

Grantees of the National Institute of Arthritis and Metabolic Diseases and the National Heart Institute have found that the mortality rate from exchange transfusions can be greatly reduced by using fresh blood treated with the anti-coagulant heparin in place of stored blood that has been treated with sodium citrate to prevent clotting. In a long-term study of 152 exchanges, performed in newborn infants with Rh incompatibility and other hemolytic diseases, the use of heparinized blood resulted in a mortality rate of 7.8 percent; when seven infants with hydrops, a condition from which survival is very rare, were excluded, the mortality rate became 1.8 percent. These rates are in striking contrast to the 13 percent reported earlier by other investigators using citrated blood.

Citrated stored blood has been implicated as the cause of many of the untoward reactions that may occur as a result of exchange transfusions in the newborn. These include convulsions, heart block, generalized irritability and even death. The enfused citrate causes a reduction in the level of calcium in the serum and spinal fluid, and the subnormal calcium levels then lead to cardiovascular irregularities and to muscle spasms (tetany). Previous reports, although limited in number, have indicated that these and other harmful effects can be circumvented by using fresh heparinized whole blood, and the present study was conducted to determine the value of heparinized blood in a large number of exchange transfusions.

All the exchanges were performed at the University of Minnesota and Minneapolis General Hospitals during the period 1945-1959. Of the total number of infants transfused, 130 were treated for Rh sensitization, 12 for ABO incompatibility, and 10 for hyperbilirubinemia (excessive bile pigment in the blood). The procedure was done in the standard way and in almost all cases through the umbilical vein. Tendency toward hemorrhage was easily controlled by subsequent injection of protamine sulfate which counteracted the anti-clotting effects of the heparin in the transfused blood. Normal clotting time was restored within fifteen minutes.

In summarizing their results, the grantees reported that in addition to the lowered mortality, it was of importance that no deaths occurred during the exchange transfusion, in contrast to the reported instances of cardiac arrest that have occurred during transfusion of citrated blood. Further, there was no evidence of irritability and tetany as may be observed when citrate is used.

"When these advantages and the data in this study are reviewed, it is seen that the use of heparinized blood with proper care

and protamine sulfate, can be utilized in the care of erythroblastotic infants to excellent advantage without the hazards associated with other anticoagulants," the investigators concluded.

The study was reported by Drs. Herschel P. Bently, Jr., Newell R. Ziegler and William Krivit, of the University of Minnesota, in the A.M.A. Journal of Diseases of Children.

RARE TYPE OF BLOOD ANTIBODY DISCOVERED

From studies of a surgical patient of the National Heart Institute who developed a severe bleeding tendency one week after operation, hematologists of the National Institute of Arthritis and Metabolic Diseases have found that there are two distinctly different types of human blood platelets, a common type and a rarer form. Individuals with the rarer form may develop antibodies against the common type following transfusion, and under certain circumstances as a result of this antibody a patient may destroy his own platelets. The study also provides a clear demonstration that different platelet types exist which are unrelated to red cell types. The significance of this finding with respect to the safety of blood transfusion is not yet fully known.

The discovery of the unusual type of antibody was made by Dr. Raphael Shulman and associates of NIAMD's Metabolic Diseases Branch, and stemmed from blood studies of a patient who developed uncontrollable internal and external bleeding one week after successful heart surgery on the clinical service of the National Heart Institute. At the time she was bleeding, blood studies revealed that all her blood platelets were gone, and on further study it was found that her blood contained an extremely high level of a circulating antibody which reacted with normal platelets and had presumably destroyed all the platelets in her blood. Because her condition was critical an exchange transfusion was carried out to remove the anti-platelet antibody. Within a 16 hour period, during which 90 percent of the patient's blood volume was replaced, all hemorrhage was stopped, and within several days thereafter her platelet count had returned to normal.

It was after the patient recovered, however, that Dr. Shulman made the peculiar finding which suggested that the woman might have an unusual type of platelet. He took a fresh sample of blood from the patient (a sample containing her newly restored platelets) and mixed it with some of the antibody-containing

blood that had been flushed out during the exchange transfusion. Since the antibody was active against normal platelets, it was assumed that it would also be active against the patient's platelets, but no reaction took place. This finding was corroborated in a second patient (hospitalized at Faulkner Hospital in Boston) with the same clinical history of hemorrhage one week after transfusion; that is, the platelets of both patients after recovery would not react with the antibody which was present in their blood at the time they were bleeding. This could have been due to some agent in the patients' blood which blocked the attachment of antibody or to the fact that the patients' platelets were different from usual normal platelets.

Geneologic studies by the NIAMD investigators of platelet type in 35 members (covering 4 generations) of the family of one of the patients as well as a general population survey of platelets from 100 randomly selected individuals indicated that the patients indeed had an unusual form of platelets and that this form of platelet is inherited as a recessive trait. Platelet typing was done using the original antibody from the patients. The unusual form of platelet was not as rare as had been expected for two percent of the population proved to have it, indicating that the chances of the usual form of platelets being transfused into an individual with the unusual form is approximately 1 in 50. It is evident that reactions are not caused every time this is done, for it was found that a patient with the rare type could receive as many as 17 transfusions with the common type without provoking antibody. In addition, there are only two other cases in the medical literature which might have had the same complication.

The NIAMD hematologic studies suggest that in order to develop an antibody and have the antibody destroy an individual's own platelets, the donor of the common type of platelets might have to be unusual also. It appears that some individuals with the common type platelet may very rarely have some of the antigen substance from their platelets free in their blood. This free substance transfused into a patient with the uncommon form of platelet may not only be necessary in order to provoke antibodies in the recipient but also may be necessary to coat the recipient's platelets and thus permit antibody to react, leading to lysis of platelets and possible severe hemorrhage.

The studies were reported to the American Society for Clinical Investigation by Drs. N. R. Shulman, R. Aster, A. Leitner, and M. Hiller.

BLOOD STUDIES PROVIDE NEW FACTS ABOUT A VITAL PROCESS

One of the most vital of all physiological processes is the binding of oxygen by hemoglobin, a process which enables red blood cells to transport oxygen throughout the circulation. Basic studies of human hemoglobin, by Dr. Makio Murayama of the Institute's Laboratory of Pathology and Histochemistry have now provided valuable new information about the mechanisms of this complex binding process. The studies were reported in the Journal of Biological Chemistry.

The objective of the research was to study the way in which hemoglobin binds heavy metals, such as mercury, arsenic and copper. The binding takes place at eight heavy metal-binding sites on each of the hemoglobin molecules, and the mechanisms of this type of binding are believed to be analogous to those of oxygen binding, which takes place at four other binding sites on the hemoglobin molecule. Thus, a full understanding of heavy metal binding can help to explain the oxygenation process. Heavy metal binding and oxygen binding can be looked upon as two sides of the same coin, since the binding sites are all close together in the molecule and influence one another.

Dr. Murayama's study of the eight heavy metal-binding sites has revealed that a cooperative interaction takes place among them. That is, once the first heavy metal ion attaches to a binding site, it becomes progressively easier for succeeding metal ions to attach to the remaining sites. The binding sites are inside the molecule rather than on its surface, and the process can be visualized as a "wedging apart" of the hemoglobin molecule. As the wedging increases it becomes easier for additional metal ions to get inside the hemoglobin molecule, attach to its binding sites, and increase the wedging effect. This type of cooperative interaction is also known to exist at the four oxygen-binding sites. At the heavy metal sites the interactions are known as mercapto-mercapto interactions (mercury-capturing interactions), and at the oxygen sites they are known as heme-heme interactions.

The NIAMD scientist has been able to determine the physical "constants" that govern the mercapto-mercapto interactions and, for the first time, measure the degree of interaction. Such measurements provide a most useful adjunct to studies of oxygenation mechanisms.

The present study also suggests an interesting approach to the treatment of sickle cell disease, a disorder caused by an abnormal type of hemoglobin known as S hemoglobin. Molecules of S hemoglobin have an abnormal shape; when they are

not carrying oxygen they appear to "stack up" inside the red blood cell, and the cell becomes twisted into its characteristic sickle shape. Such sickle shaped cells seriously impair blood flow in the capillary network and account for the pathology of the disease. The apparent stacking does not occur as long as S hemoglobin milecules are wedged apart, either by oxygen molecules or by heavy metal ions, so that sickling could be prevented if heavy metals were kept bound to the hemoglobin. Unfortunately, the heavy metals are too toxic for this purpose. What is needed, therefore, is some innocuous material that would attach to the heavy metal-binding sites and keep the S hemoglobin molecule wedged apart. Studies are being continued in an attempt to find a material that might accomplish this purpose.

GAMMA GLOBULIN EFFECTIVE AGAINST POST-BURN INFECTIONS

Studies of burned children in Lima, Peru, have shown that injections of gamma globulin may reduce the incidence of deadly post-burn infections by nearly one half, a National Institute of Arthritis and Metabolic Diseases' scientist reported to the First International Congress on Research in Burns. Dr. Nicholas A. Kefalides of the Laboratory of Pharmacology and Toxicology reported the successful results of a continuing study of burns and their treatment which has been carried out in both NIAMD laboratories and the Children's Hospital in Lima.

The gamma globulin treatment was developed to counteract the overwhelming infection usually caused by Pseudomonas aeruginosa, a microorganism that is resistant to all commonly used antibiotics. In instances of severe burns the primary cause of death is burn shock; however, many times patients survive the shock period only to succumb to the bacterial infection within several days.

Earlier NIAMD animal studies by Drs. R. C. Millican and S. Rosenthal indicated the value of gamma globulin, the blood serum fraction containing antibodies, in combatting the post-burn infection. Clinical trials of gamma globulin therapy began in 1957 on burn victims at the Lima hospital and the value of the treatment has now been established in over 100 cases.

In a control group of 111, the incidence of septicemia (generalized blood infection) was 41 percent. In the 104 gamma globulin patients the septicemia was 21 percent. Patients received approximately 60 ml. of gamma globulin. Treatment was most effective in children under 4 years old with burns under 25 percent of the body area. In older children, although the gamma globulin treatment significantly lowered the incidence of septicemia, it had considerably less effect on total late mortality.

"The results indicate that antibiotic prophylaxis does little to prevent wound infection or septicemia, while gamma globulin prophylaxis significantly reduced septicemia and mortality in young children," Dr. Kefalides reported.

PHYSICAL BIOLOGISTS UNCOVER NEW FACTS ABOUT PHOTOSYNTHESIS

Studies by physical biologists at the National Institute of Arthritis and Metabolic Diseases are providing important new facts about photosynthesis. Of considerable interest is the structure of the chloroplasts, the small green bodies in higher plants and green algae which contain chlorophyll, the major photosynthetic pigment. In the Section on Photobiology, headed by Dr. F. S. Brackett, NIAMD scientists are developing new techniques and instruments to uncover how the chloroplasts capture the energy of sunlight and use it to produce organic compounds from carbon dioxide and water.

Using a technique known as absorption microscopy, Drs. R. A. Olson and C. L. Greenblatt, and E. K. Engel, have been able to confirm--by light microscopy--what had been previously suggested by electron microscope studies. This was that chlorophyll exists in a number of layers inside the living chloroplasts. This lamellar structure, a "sandwiching" of a layer of single molecules of chlorophyll with layers of protein and lipid, is believed to be an aspect of the essential structural characteristics of the chloroplasts which enables them to convert light energy to chemical energy. The work was reported in The Journal of Biophysical and Biochemical Cytology.

Dr. Olson and his NIAMD associates have also constructed a device to measure the emission spectra of Chlorella, a simple chlorophyll-containing alga. These plants, like all photosynthetic ones, fluoresce when excited by light of particular wavelengths (in this case 4358 A), and the characteristics of this emitted light help to identify the metabolic changes taking place in the cells. The new instrument, known as a rapid scanning microspectrofluorimeter, can scan the spectra of the emitted light as rapidly as once a second. It will be described in detail in the August issue of The Review of Scientific Instruments.

These NIAMD studies of photosynthesis, only a small part of the Institute's total research in physical biology, have produced three valuable results. First, the studies provide a means for studying energy transfer in a biological system where the input energy (light) can be measured exactly; second, they provide a convenient system for studying the synthesis of porphyrin compounds, compounds which include, in addition to chlorophyll, the heme in hemoglobin as well as the various cytochromes that play a vital role in cell metabolism; and third, the studies are leading to the development of the very specialized tools needed for biophysical research. In the near future these same tools may find valuable application to many problems of human cellular metabolism.

ADVANCED ELECTRONIC TECHNIQUE USED TO STUDY ATOMIC STRUCTURE

Physical biologists at the National Institute of Arthritis and Metabolic Diseases have used an advanced electronic technique known as nuclear magnetic resonance to study the atomic structure of porphyrins, complex molecules found in hemoglobin, chlorophyll and other important biological materials.

The porphyrins are pigments which occur throughout the plant and animal world. They form the base of chlorophyll in plants and hemoglobin in man and readily combine with metals such as iron and magnesium. The porphyrins are of fundamental importance to many oxidation-reduction reactions in the body and are also known to affect radiation sensitivity. Persons afflicted with one of the forms of porphyria, a rare disorder of porphyrin metabolism, are sensitive to light and must avoid direct exposure to the sun. In radiation research certain porphyrin compounds have been used to protect animals against lethal doses of radiation.

The NIAMD study was done to learn more about the atomic structure of these important molecules and utilized nuclear magnetic resonance (NMR) equipment. The study was reported by Dr. Edwin D. Becker and Robert B. Bradley of NIAMD's Laboratory of Physical Biology in the Journal of Chemical Physics.

The NMR technique of the physical biologists makes it possible to determine the presence or absence of certain functional groups (OH, NH, etc.) in a molecule and to determine the relative positions of these groups, thus giving a three dimensional picture of molecular structure. The test substance is placed in a strong magnetic field and bombarded with radio waves which can be varied in frequency. Once in the magnetic field, the

molecules of the sample substance absorb radio waves of certain frequencies, the particular frequencies being determined by the atomic structure of the molecule. By noting what frequencies are absorbed, the investigators can determine what atomic particles are present and how they are arranged.

NMR analysis of several non-metallic porphyrin compounds revealed that their NMR spectra had considerably different values from those that had been expected. The NIAMD scientists found that the differences could be accounted for in large part by the presence of a "ring current" around the porphyrin molecule. The molecule has a ring-like structure and the current is due to flow of electrons around the periphery of the molecule. Such a current would produce a small magnetic field that would interact with the larger field of the NMR equipment, and this would account for the unexpected results.

The finding is of considerable interest to other NMR spectroscopists for it will explain what would otherwise be confusing experimental results. Additionally, the NIAMD study has indicated that some unknown structural factor may also be involved, suggesting that the presently accepted "model" of the porphyrin molecule is inadequate. Further studies to clarify this point will be done on samples of metal-containing porphyrins.

The porphyrin samples used in the study were provided by Dr. Cecil J. Watson, Professor of Medicine at the University of Minnesota Medical School, who is chairman of NIAMD's Board of Scientific Counselors. Dr. Watson has specialized for many years in the study of porphyria and spent several months at the Institute last year as a Visiting Scientist.

PROGRAM DEVELOPMENT

WORLD NUTRITION CONGRESS DRAWS 2000 SCIENTISTS TO WASHINGTON

Over 2000 nutrition scientists participated in the week-long Fifth International Congress on Nutrition which was held in Washington, D. C., and opened by President Eisenhower. During the Congress, the first to be held in the U. S., experts from 65 nations reported on nutritional progress throughout the world and discussed the food problems that still exist in many areas. Two of the interesting highlights of the international meeting were reports that underproduction of food, not overpopulation, will be the major nutritional problem facing the world for the next 40 years; and that in the United States the worst nutritional problem is overeating.

A number of special panel discussions were held on such subjects as the evaluation of nutritional status in man, nutrition in maternal and infant feeding, the effects of processing and additives on foods, and the new possibilities in nutrition research. In addition, there were many individual study sections. These covered a whole spectrum of nutrition studies from proteins and amino acids, to the effect of bile acids on plasma cholesterol.

As well as the great number of important basic scientific studies that were reported at the Congress, there were also some of immediate practical value, such as new and effective means of preventing protein deficiency on a population-wide scale. This is one of the most acute nutrition problems existing in some of the underdeveloped areas of the world; in Africa and Central and South America it accounts for the high incidence of the disease known as kwashiorkor, a protein malnutrition syndrome which often leads to death and occurs in children who are fed an almost exclusive starch diet. One successful solution to this problem has been developed by the Institute of Nutrition for Central America and Panama (INCAP). Under grants from the National Institute of Arthritis and Metabolic Diseases, INCAP scientists have developed a vegetable protein formula which has a well balanced amino acid profile and, most importantly, is based on locally produced agricultural products. The special high protein diet is called Incaparina and contains 38 percent cottonseed meal, 29 percent corn meal, 29 percent sorghum meal, 3 percent yeast, and 1 percent calcium carbonate. The diet has proven extremely valuable in preventing and treating kwashiorkor, and this classic achievement by INCAP points the way to similar economically feasible solutions to the problem of protein deficiency in other undernourished regions of the world.

Staff members of the National Institute of Arthritis and Metabolic Diseases (NIAMD) presented eight papers and participated in committee and other functions of the Congress. A total of 18 papers were given by the National Institutes of Health staff, with the largest number, four, devoted to research with germfree animals.

The Congress was sponsored by the International Union of Nutritional Sciences, the American Institute of Nutrition, and the National Academy of Sciences--National Research Council. It was supported, in part, by the NIAMD, the National Cancer Institute, and the National Institute of Dental Research.

NATURAL HISTORY OF DIABETES SUBJECT OF LARGE NEW STUDY

The first large-scale, carefully controlled and long-term study of the natural history of diabetes is now in its pilot stage at seven research centers located throughout the country. Under grants from the National Institute of Arthritis and Metabolic Diseases (NIAMD), the new University Group Diabetes Study will investigate the incidence and severity of late diabetic complications such as retinopathy, atherosclerosis, kidney and nervous system disorders, in large groups of reasonably controlled patients treated in various ways. Such diabetic complications have long been of great concern to the medical profession; diabetes is, for example, numerically the greatest cause of blindness in the United States.

The study, originally conceived as an investigation of the effects of oral hypoglycemic agents (drugs which reduce blood sugar levels) on diabetes, has been designed to determine the rate of development and severity of late diabetic complications and to determine if this rate is in any way affected by several therapeutic measures involved in the study on a long-term basis.

The therapeutic measures being used to control diabetes are: (1) special diet, (2) special diet plus the oral drug tolbutamide, (3) special diet plus insulin given in dosage to maintain good chemical control of blood sugar levels, (4) special diet plus a fixed dosage of insulin administered on the basis of body weight.

The first year of the NIAMD-sponsored study is regarded as a trial run and patients selected for the pilot stage will be disqualified when the full-scale investigation gets underway early in fiscal year 1962. Approximately 700 patients, 100 at each center, will be included in the study, and the patients will be selected from those diabetics whose disorder has been diagnosed within six months prior to inclusion in the study, and who have had no previous

therapy. In addition, their disease must be controllable by diet alone and they must have a life expectancy of at least five years. Patients are subjected to batteries of tests at the centers on a repeating basis. After careful instruction, they will run certain of their own tests, administer their own drugs, check their own diet and record all data in individual diaries.

Seven principal investigators, an eminent epidemiologist and some 25 of their associates now compose the group. These investigators are: Dr. Max Miller (chairman), University Hospital, Western Reserve University, Cleveland; Dr. Christian R. Klimt (group epidemiologist and chief of the coordination center), School of Public Health, University of Minnesota; Dr. Richard A. Field, Massachusetts General Hospital, Boston; Dr. Frederick C. Goetz, Medical School, University of Minnesota; Dr. Martin G. Goldner, Jewish Chronic Diseases Hospital, Brooklyn, New York; Dr. Charles A. Jones, Williamson Memorial Medical Center, Williamson, West Virginia; Dr. Harvey C. Knowles, Jr., University of Cincinnati, Department of Internal Medicine, Cincinnati General Hospital, Ohio; and Dr. Thaddeus E. Prout, School of Medicine, Johns Hopkins University, Baltimore. Dr. James W. Pratt, NIAMD, serves as liason officer to the group.

In addition to NIAMD funds, the group receives support from the individual investigators' own institutions. The Upjohn Company, Eli Lilly & Company, and the Ames Company, Inc. have agreed to provide drugs and products for the study without having a voice in the management of the study. The trials were approved by the NIAMD Advisory Council for a period of seven years and are forecast to run for a minimum of ten. If successful, the study could become a prototype for large-scale cooperative clinical trials in other disorders.

HIGHLIGHTS OF PROGRESS

IN DENTAL RESEARCH

1960

Items of Interest on Program Developments and Research

Studies Conducted and Supported by the National Institute

of Dental Research

DENTAL CARIES

MECHANISM OF DENTAL DECAY STUDIED IN GNOTOBIOTIC ANIMALS

Previous studies at the University of Notre Dame using germfree rats fed a cariogenic diet provided dental scientists for the first time with unequivocal evidence that experimental caries do not occur in the absence of bacteria. Following this discovery attention was logically directed toward isolating specific decay producing microorganisms in gnotobiotic animals. These studies soon led to the establishment of a direct cause and effect relationship between dental decay and an enterococcus (reported from Lobund) and a single strain of oral streptococcus reported by NIDR scientists.

In studies this year by Drs. Robert J. Fitzgerald and Harold V. Jordan, Laboratory of Microbiology, and Dr. Harold R. Stanley, Clinical Investigations Branch, 16 weanling white rats of the Lobund germfree strain were divided into two groups and housed in Reyniers' germfree tanks. Animals in group I were monoinfected with a streptococcus isolated from the oral cavity of a caries-active rat, while those in group II remained as germfree controls. A third group of rats used as conventional controls were offspring from breeders of the Lobund germfree strain, brought to a conventional environment prior to mating. Animals in all three groups were maintained on the same cariogenic diet that was autoclaved and supplemented with vitamins.

Each of the rats in the conventional control group, sacrificed after 100 days on the cariogenic diet, and all animals in the monoinfected group, sacrificed after 77 days, showed extensive

cavitation frequently involving both the dentine and pulp chamber of molars. In contrast, the teeth of the germfree animals, sacrificed after 84 days, were free of caries in spite of considerable food impaction and occasional fracture of a molar cusp.

These significant studies with gnotobiotic animals have extended the knowledge of oral disease by demonstrating the etiological role of the streptococcus organism in the production of experimental caries.

DENTAL SCIENTISTS SEEK CARIES INHIBITING AGENTS

As previously mentioned germfree animals have clearly established a causal relationship between certain oral bacteria and dental caries. While antibiotics are effective in depressing the oral flora, certain undesirable features such as sensitivity, and development of antibiotic resistant organisms limit their practical use. On the other hand, chemical agents which interrupt or alter the metabolism of oral bacteria may provide a rational approach to the problem. Thus, investigators in the Laboratory of Microbiology reported this year on experimental studies with carbonyl-binding compounds as examples of potentially useful agents.

Sodium metabisulfite, a carbonyl-binding agent, has been shown to inhibit experimental caries in rats. Two other carbonyl-binding agents, CMA (carboxylmethoxylamine hemihydrochloride) and dimedone were also tested and found active. In studies conducted by Drs. H. V. Jordan, R. J. Fitzgerald, and Miss N. D. Berger, these three compounds were tested separately in laboratory rats maintained on a cariogenic diet. Results showed that an 82 per cent reduction in caries score was obtained when CMA was fed in the diet at a level of 0.004 molar for the first week and 0.002 molar for the remainder of the 90 day test period. Feeding of 0.016 molar sodium metabisulfite resulted in an inhibition of 86 per cent, while rats fed the aldehyde-binding agent dimedone at a level of 0.029 molar showed a 55 per cent reduction in caries score. In vitro experiments showed that the relative cariostatic effects of these three compounds generally paralleled their growth-inhibiting properties for a variety of microorganisms including lactobacilli, streptococci, staphylococci and diptheroids.

These continuing studies of inhibiting agents, coupled with recent contributions defining the production and transmission of caries, and the identification of specific oral pathogens, are providing valuable new information necessary for a practical approach to the prevention and control of dental decay.

NEW METHOD FOR THE ASSESSMENT OF CARIOSTATIC AGENTS

The screening of potential caries-inhibitory agents in experimental animals is an important prerequisite to clinical trials in humans. Recent progress in studies of the production and transmission of dental caries in hamsters has led to the development of new and improved procedures for the rapid assessment of caries-inhibiting agents in animals.

Conventional screening methods for assessing cariostatic agents are unsatisfactory in that they are limited to the recording of caries initiation and progression of lesions. In demonstrating a new method to assess cariostatic agents, Dr. Paul H. Keyes, Laboratory of Histology and Pathology, selected young albino hamsters known to be infected with a cariogenic flora and maintained them on a caries-test diet for a period of 10-14 days. At the end of this time carious lesions were detectable in the first and second molars; the third molars not yet being erupted. Following this evidence of caries activity, the agent to be tested for cariostatic properties was introduced to the animal via the diet or drinking water. The caries-inhibiting effect of the drug was then determined by noting the degree of arrest of the previously induced lesions in the first and second molars and the degree of prevention of caries in the newly erupted third molars. Finally, withdrawal effects of the agent could be determined by observing the reactivation of caries in arrested lesions and the initiation of new cavities in the previously protected third molars. In the described assessment procedure parallel observations on untreated control animals maintained on the caries producing diet but not receiving any caries-inhibiting agent, showed a rapid progression to severe caries within the test period of 42 days.

Collaborative efforts are now under way with Dr. R. J. Fitzgerald, Laboratory of Microbiology, to trace these events microbiologically. Further studies may delineate more clearly the basic mechanisms involved in the pathogenesis of dental caries.

INVESTIGATORS STUDY MODE OF CARIOSTATIC ACTION OF TETRACYCLINE

Previous studies with tetracycline have shown a characteristic deposition of this antibiotic in the bones and teeth of animals. Moreover, caries inhibition has been achieved by the addition of tetracycline to the diets of test animals. NIDR scientists have now reported on studies to investigate the possible relationship between skeletal deposition and residual cariostatic effects. In laboratory experiments by Drs. I. Zipkin and Rachel Larson, Laboratory of Biochemistry, Sprague-Dawley rats were bred and

subsequently divided into four groups. Animals in groups A, B, and C received 2.5 mg/ml of tetracycline hydrochloride in drinking water, and group D received distilled water. Group A animals received the antibiotic during gestation only; group B during gestation and for 14 days following birth; and group C during gestation, lactation, and the remainder of the preweaning period. Group D animals received distilled water until weaning at 21 days of age. A standard laboratory diet was available during gestation and lactation and a non-cariogenic diet was offered thereafter until weaning. After weaning, the offspring of animals in all four groups received distilled water and a caries test diet for 60 days.

Tetracycline did not adversely affect litter production, weight gains, diet intakes or fluid consumption in either mothers or offspring. The most striking effect of tetracycline was seen in the marked reduction in caries activity under all experimental regimens. For example, the caries scores for offspring in the three experimental groups were 2.3, 4.5, and 1.8 respectively, and in the control group was 20.3. Although characteristic fluorescence was seen in the teeth and bones, thereby indicating the presence of tetracycline, the low level of caries activity could not be related directly to the incorporation of the antibiotic in the teeth. Rather, tetracycline appears to act by depressing the intestinal microflora and oral flora which had previously been demonstrated by NIDR scientists to contain the caries activating organisms.

PHOSPHATE-DENTAL CARIES STUDIES FOCUS ATTENTION ON THE CHEMICAL REACTIVITY OF TOOTH SURFACES

There has been accumulated in recent years convincing evidence that dental decay in experimental animals is dramatically reduced by adding certain mineral phosphates to the diet. This finding provided a firm foundation for clinical studies now under way to assess the inhibitory effects of dicalcium phosphate on human caries. Concurrent with these laboratory and field trials are studies by Dr. F. J. McClure, Chief, Laboratory of Biochemistry, to identify the mode of inhibitory action associated with mineral phosphates.

Dr. McClure has reported evidence that cariostatic effects of the phosphate minerals occur primarily within the oral cavity rather than via an indirect systemic route. For example, it was shown that the relative solubility of phosphates is an important factor, i. e., highly insoluble forms, particularly sodium and ammonium phosphate, are extremely effective. Further support of this hypothesis was seen when insoluble dicalcium phosphate proved to be effective only if sodium chloride was present in the diet to

serve as a solubilizing agent. In addition, when Na_2HPO_4 was intubated, thereby bypassing the oral cavity, it was not as cariostatic as when consumed as part of the diet. Just how the cariostatic effects of the phosphate minerals occur remain speculative. However, data gathered to date suggest a direct action of the mineral on oral enamel surfaces thereby focusing attention on the chemical reactivity of tooth surfaces. Paralleling these considerations is the nutrition of the oral flora and their metabolic products which may undergo significant change in the presence of freely available phosphate ions.

GRANTEES DEMONSTRATE CHEMICAL ACTIVITY OF SUCCINIC ACID IN ENAMEL DECALCIFICATION

Lactic acid has traditionally been suspected as the chief causative agent in demineralization leading to dental caries. NIDR grantees reported this year on <u>in vitro</u> studies which showed that, in concentrations found in the mouth, succinic acid is significantly more active than lactic acid in the dissolution of enamel.

In investigations by Mr. W. Wynn, Dr. J. Haldi, and Miss Law, 60 caries free human teeth were extracted, cleaned and the roots covered with acrylic leaving only the enamel surfaces exposed. In order to compare the chemical activity of lactic and succinic acids, ten teeth were suspended in each of six beakers and covered with 250 ml. of one of six different solutions. The first three solutions were each 0.1 per cent lactic acid, buffered with dilute sodium hydroxide to pH 4, 5 and 6, respectively. The remaining three solutions were 0.1 per cent succinic acid, also buffered to pH 4, 5 and 6 with dilute sodium hydroxide. The beakers were then incubated at 37°C. Every 24 hours the solutions were renewed. Each day a quantity of solution was removed and saved until at the end of 2 weeks, 3.5 liters of each of the six had been collected. The six collections were then evaporated, residues reduced to ash, and analyzed for calcium, phosphorus and magnesium.

Results showed succinic acid to be considerably more active than lactic acid in removing calcium, phosphorus and magnesium. For example, at pH 5 succinic removed 24 mg. Ca, 11.5 mg. P and 320 mg. of Mg as compared with lactic which removed 7 mg. Ca, 3 mg. P and 15 mg. Mg. Thus, in this pH range succinic removed approximately five times as much Ca and P as lactic acid and up to twenty times as much Mg.

These studies have encouraged further investigations of the origin of succinic acid and other Krebs cycle acids in saliva and their role in <u>in vivo</u> enamel decalcification.

PERIODONTAL DISEASE

TARTAR FORMATION NOT DEPENDENT ON MICROORGANISM

Previous studies at NIDR have demonstrated the presence of tartar-like deposits in germfree mice. This observation was recently confirmed in studies which showed, with X-ray diffraction analyses, that the crystalline component of tartar formed on the molar teeth of germfree rats was identical to that seen in conventional animals and in humans.

In experiments by Dr. R. J. Fitzgerald, Laboratory of Microbiology, and Mr. E. G. McDaniel, National Institute of Arthritis and Metabolic Diseases, six rats of the Lobund strain were maintained in a germfree environment for 90 days. Periodic bacteriological checks on the food and excreta of the animals confirmed the absence of viable microorganisms during the test period. At termination of the experiment the animals were sacrificed and a quantity of tartar-like material from first molars was collected and pooled for X-ray diffraction studies. The presence of crystalline apatite was detected and, thus, the mineral portion of the germfree calculus was shown to be the same as that in calculus from conventional animals and from humans.

In demonstrating that the formation of typical calculus does not depend on the presence of living or dead microorganisms, these studies have further explained earlier findings by NIDR scientists that calculus will develop in conventional rats whose oral flora was depressed by antibiotics.

ORAL EFFECTS OF ANTIBIOTICS ARE STUDIED

One of the continuing research programs of the Dental Institute is concerned with the testing of antibiotics and other agents which may be used for the control of calculus formation, caries and other oral conditions.

Scientists have recently completed studies in which chloramphenicol and chlortetracycline, both broad spectrum antibiotics, were tested for their effect on the development of dental calculus and caries in rats. The drugs were added at a level of 0.005 per cent to one diet known to promote caries development and to another diet known to accelerate calculus deposition. Calculus severity ratings were then compared with control groups receiving the same diets without antibiotics. The results indicated that neither antibiotic influenced calculus formation. Both, however, demonstrated significant caries reduction in the test animals, with chlortetracycline showing the more marked effect. These results complement the earlier NIDR findings with germfree animals which showed that calculus-like deposits may occur in the absence of oral bacteria while dental decay

does not. In the current study further significant findings were made when samples of the crude and ashed calculus deposits from the animals were analyzed and subjected to electron and X-ray diffraction. The mineral phase was identified as hydroxyapatite; thus, the dental deposits in the rat may be considered analogous to oral calculus of humans.

Data from this cooperative study by Drs. R. J. Fitzgerald, H. V. Jordan, D. B. Scott and Mr. H. G. McCann, representing three Laboratories of the NIDR, will provide research workers with a useful tool for the study of deposition and control of dental calculus under experimental conditions.

GRANTEES INVESTIGATE CALCULUS MORPHOLOGY

Dental calculus and the mechanism involved in its formation are matters of widespread interest because of their combined role in periodontal disease and because of the similarity of calculus to kidney stones, teeth and bone. Two dental grantees at Harvard University reported this year on electron microscope studies of calculus and some of the factors involved in its development.

Dr. F. Gonzales, Senior Research Fellow, National Institutes of Health, and Dr. R. F. Sognnaes, formerly Professor of Oral Pathology, Harvard School of Dental Medicine, studied microthin calculus sections using electron microscopy and electron diffraction. The resulting micrographs showed occurrence of two types of calcification fronts -- one apparently representing an older and more mature concretion -- the other showing actively growing and incompletely mineralized early calculus.

Throughout the mass were found the microorganisms typically seen in calculus. Some organisms were completely entombed within solidly calcified areas and were seen in various stages of degeneration. In addition, mineral crystals were observed in the regions between organisms, in the spaces occupied by degenerated bacteria and within some bacteria themselves. Many of these inorganic crystals were of the same size and shape as those in bone. While microorganisms have previously been implicated in calculus formation, these observations graphically indicate for the first time that oral bacteria may themselves be subject to mineral deposition. Continuation studies in this field are designed to explore calculus formation and deal with the developmental stages of the calcification phenomena, as well as histochemical analyses of the substances involved.

SUSCEPTIBILITY OF GINGIVAL TISSUE TO ENDOTOXINS FROM ORAL BACTERIA IS DEMONSTRATED

The finding that certain oral bacteria possess endotoxic properties that may contribute to the pathogenesis of periodontal disease has been demonstrated in studies by investigators in the Laboratory of Microbiology. In previous experiments using endotoxins from oral bacteria, primary toxicity to rabbit skin was demonstrated, and the local Shwartzman reaction of hemorrhagic necrosis was produced by intradermal and intravenous injections of endotoxic preparations. Recognizing the possibility that such endotoxins may have etiologic significance in oral soft tissue diseases, either through primary toxicity or through Shwartzman-like effects, one of the recently isolated endotoxins was tested in rabbit oral mucous membrane. In a study conducted by Drs. A. A. Rizzo and S. E. Mergenhagen, 200 micrograms of a purified endotoxin (lipopolysaccharide) from Veillonella organisms was injected into the gingiva and palatal mucosa of rabbits. Control injections of saline, as well as lipid-carbohydrate-protein products isolated from viridans streptococci, were given at corresponding sites on the opposing sides of the mouth. To provoke the Shwartzman reaction, rabbits were given 125 micrograms of Veillonella endotoxin in the marginal ear vein 12-24 hours following local injection. Six hours after intravenous injections animals were sacrificed and processed for histological examination.

Results showed that in rabbits receiving preparatory and provoking injections of endotoxin, gross and histological signs of the classical Shwartzman reaction were evident in the oral mucosa. Control sites given streptococcal products and provoked with intravenous Veillonella endotoxin did not produce this reaction. This demonstration of classic endotoxins in oral bacteria, and the marked susceptibility of gingival tissue to these toxins, offer a better understanding of the inflammation sequence characterizing periodontal disease.

RICE RAT SHOWN HIGHLY SUSCEPTIBLE TO PERIODONTAL DISEASE

Gross observations of periodontal lesions in the rice rat have revealed the susceptibility of this species to periodontal disease. Grantees reported this year on detailed histologic studies that identify the stages of progression of periodontal disease in these animals.

Dr. Om P. Gupta, University of Illinois, and Dr. James H. Shaw, Harvard University, bred 75 rice rats under controlled conditions maintaining them on a purified ration for a period ranging from 8 to 30 weeks. The jaws were X-rayed, examined grossly and then serially sectioned and stained. Subsequent histologic examinations enabled the identification of susceptible and resistant strains. Results showed that 62 per cent of the animals were susceptible to periodontal disorders. In addition, the animals in one susceptible

litter showed extensive lesions as early as 11 weeks of age, while resistant litters failed to show any appreciable pathology even at 27 weeks. Periodontal lesions were characterized by inflammatory cells and edema in the epithelial and subepithelial layers, dilatation and engorgement of blood vessels, connective tissue edema, periodontal membrane thickening and crestal resorption. About twenty per cent of the susceptible animals showed migration, degeneration and necrosis of gingival and periodontal fibers, and extensive bone resorption.

These studies suggest that the inordinate high susceptibility to periodontal disease in the rice rat is associated with genetic factors, and further establish the unique importance of this animal in periodontal disease research.

CLINICAL INVESTIGATIONS

ORAL ULCERATED LESIONS STUDIED IN LARGE POPULATION GROUPS

Herpes labialis (fever blisters) and aphthous stomatitis (canker sores) are both recurrent painful ulcerations of the mucous tissues of the mouth. A study of these disorders as they occur in a large population group was reported.

The purpose of the investigation, involving some 1800 persons, was to determine the prevalence of and patient experience with aphthous and herpetic lesions. Participants in the study directed by Dr. I. I. Ship included the student bodies of the Schools of Medicine, Dentistry, Dental Hygiene, Veterinary Medicine, Graduate Nursing, and Hospital Nursing of the University of Pennsylvania. Also participating were nursing students at the Philadelphia General Hospital and the Presbyterian Hospital. A specially designed questionaire was employed, and all responses were checked for accuracy by comparison with answers to similar questions on completed medical history forms.

Analysis showed that 38 per cent of the students had a history of recurrent herpes labialis with no sex differences noted. On the other hand, a significant sex difference was seen in the 57 per cent of females and 52 per cent of males that had aphthous ulcerations. In addition these prevalence levels were considerably above those previously reported in population studies by other investigators. Age, racial background, marital status, school, and class differences were not seen in either disease, and students susceptible to episodes of aphthous stomatitis experience more frequent recurrences than those susceptible to herpes labialis. Of particular interest to investigators was the strong relationship noted between emotional factors and aphthous ulcerations. This was seen in both sexes and appeared to be related to the severity of the disease.

NIDR CLINICAL INVESTIGATOR JOINS SURVEY TEAM IN CHILE

The Interdepartmental Committee on Nutrition for National Defense conducted a nutrition survey in Chile this year. Dr. Carl J. Witkop, Jr., Chief of the Human Genetics Section, Clinical Investigations Branch, served as dental investigator on the special team composed of 14 American scientists and 14 Chilean health officers.

During the period of study approximately 18 locations were visited, where food samples, dietary histories, and blood and urine samples were collected for analysis. In addition, a total of 1900 dental and 10,000 medical examinations were made.

Data collected from dental-medical examinations will complement those recently obtained from similar epidemiological studies in other foreign countries where ethnic, geographic, and climatic conditions as well as dietary habits vary widely and are in marked contrast with those seen in this country. Significant among the findings in Southern and Central Chile was the relatively high incidence of both caries and periodontal disease, the latter being associated with the prevalence of heavy calculus deposits. In contrast, it was noted from examinations made in North-central Chile, including the city of La Serna, that caries and periodontal disease were notably less prevalent, the former being associated with a high incidence of dental fluorosis confined to that area.

In observations of oral congenital anomalies it was found that non-fluoride white opacities of the teeth occurred less frequently in the Chilean population than in comparable U. S. population groups. In addition, Torus Mandibularus, a bony outgrowth on the inside of the mandible, seen in about 15 per cent of U. S. populations, was found in only one individual in those examined in Chile. Torus Palatanius, a bony overgrowth of the hard palate, common in this country, was rarely seen in Chile.

GENETICIST DESCRIBES HEREDITARY DISEASE

Dr. Carl J. Witkop, Jr., recently described a rare hereditary disease of the oral and ocular tissues before members of pathology and bacteriology groups meeting this year in Memphis, Tennessee.

Speaking before a joint meeting of the International Academy of Pathology and the American Association of Pathologists and Bacteriologists, Dr. Witkop reported that the genetic syndrome occurred principally among a collection of family groups in North Carolina and that it appeared to be similar to, and perhaps identical with, the white sponge nevus described by Dr. A. B. Cannon in 1935.

The Cannon type normally involves the vaginal and anal mucosa; however, these sites were not affected in the group surveyed by Dr. Witkop with Dr. John B. Graham and Mr. Clemm R. Shankle of the Department of Pathology, University of North Carolina Medical School, and other investigators from the Ophthalmology Branch of the National Institute of Neurological Diseases and Blindness.

Termed as Hereditary Intra-epithelial Dyskeratosis, the disease was characterized by lesions on the buccal mucosa, floor of the mouth, side of the tongue, as well as the bulbar conjunctivae. History, physical findings, histological appearance and histochemical reactions supported the belief that both oral and ocular symptoms were local manifestations of the same disease process. There were some indications that the syndrome was subject to seasonal exacerbations. Careful examination, however, failed to reveal any evidence that it was due to a local geographic factor such as an allergen or occupational hazard. Other data also tended to eliminate viruses, vitamin deficiencies and microbial infections as the responsible factors.

NIDR GRANTEES REPORT WOUND HEALING ACCELERATION BY DILANTIN SODIUM

Hyperplasia of the oral gingiva is a common reaction among epileptics following systemic administration of sodium diphenylhydantoinate (dilantin sodium). To explore the theory that the compound might accelerate the healing of gingival and other wounds through enhanced collagenization, a controlled study of the effect of dilantin sodium on the tensile strength of healing wounds was carried out by grantees at the Indiana University School of Dentistry. The study, as reported by Drs. W. G. Shafer, R. E. Beatty, and W. B. Davis, consisted of three groups of 60-day old male albino rats, each group subdivided equally to serve as experimental and control. Experimental animals in group I received 50 mg per day of dilantin sodium by stomach tube. Experimental animals in groups II and III received 25 mg per day dissolved in special diluent and injected intramuscularly. Control animals received no drug, and all animals participating in the study were maintained under identical conditions of diet and quarters.

Dilantin sodium was administered for 18 days before induction of experimental wounds in both experimentals and controls. Three to four days postoperatively, the tensile strength of wounds was tested on strips of tissue that had been cut at right angles to the initial incision. With one end of the strip held stationary, bromoform was added to a cup suspended from the other end of the tissue strip, and values were recorded as ml of bromoform required to cause separation of the wound. Findings in the respective three experimental groups were 147, 127, and 76 per cent greater than those in the companion control groups.

Thus, it was demonstrated that the systemic administration of dilantin sodium produces a dramatic increase in tensile strength of healing wounds in rats and appears to be the same basic reaction that accounts for gingival hyperplasia in epileptics receiving this anticonvalescent drug.

MALOCCLUSION AMONG NEGRO CHILDREN IS STUDIED

The incidence of malocclusion in the North American Caucasian population is well documented; however, comparable data on the Negro population, which now comprises about 12 per cent of the total United States population, have not been reported. Therefore, a survey of Negro children was undertaken this year by NIDR grantees at Howard University.

Malocclusion of the teeth not only interferes with the normal function of mastication, but also causes facial disharmony and invites dental disease. Thus, to render a more complete national profile of this dental abnormality, a survey of Negro children in the Washington, D. C. area was undertaken by Dr. L. A. Altemus of Howard University College of Dentistry in conjunction with the District of Columbia Health Department.

A total of 3500 area children were examined from which 3298 were selected for study. These children ranged from ages 12-16, each had acquired permanent teeth and none had received orthodontic treatment during their lifetime. The test group, selected from four junior and senior high schools, included a cross section of all income groups and represented the great racial mixture of the North American Negro. The frequency of occurring malocclusion was determined by the widely recognized Angle Method of classification and by counting the teeth that were out of normal alignment.

Tabulation of data showed the frequency of malocclusion to be high in the Negro child population of this country. Approximately 83 per cent had improper occlusion of teeth, while 4 per cent had nearly perfect occlusion. Thirteen per cent had teeth slightly malpositioned, but not requiring orthodontic treatment. There were approximately 6 malocclusions per child and no significant difference in incidence between sexes was noted. With the one exception that Caucasian children averaged ten malocclueded teeth per child, these data compared closely with the frequency of occurring malocclusion in the Caucasian child population reported by other investigators.

BIOCHEMICAL STUDIES

APPARENT DIFFERENCE IN EXCRETION OF CALCIUM AND RADIOCALCIUM IS REPORTED

During the course of studying the excretion of certain minerals in rats, NIDR investigators found evidence of unexpected variability in the renal clearance of labeled and unlabeled calcium. These findings of possible significance to metabolic studies employing radiocalcium as a tracer were reported this year. In experiments by Dr. Robert Likins and Miss Doris Craven, Laboratory of Biochemistry, weanling rats (Group I) were injected intraperitoneally with a solution containing Ca^{45} and a trace amount of unlabeled calcium. Urine was collected at the end of 30 minutes and 60 minutes, and analyzed for radiocalcium and total calcium. One hour after injection, blood from each animal was also analyzed for Ca^{45} and Ca^{40}. A comparable group of rats (Group II) were denied food for 12 hours and the experiment repeated.

Results showed that in the two groups of animals the per cent of the injected dose of labeled calcium excreted in the urine was significantly less in the case of the starved rats. On the other hand, the total amount of calcium excreted remained the same in starved and unstarved animals. While a slower absorption of the injected Ca^{45} might logically account for this diminished excretion by Group II rats, additional experiments showed that plasma of similarly starved rats contained as much radiocalcium 30 minutes after injection as did non-starved controls.

Although the explanation for this discrimination against radiocalcium is not readily apparent, there is no reason to believe that the renal clearance rates of Ca^{45} and Ca^{40} differ intrinsically. A possible explanation may lie in a different partitioning of the injected label among the body calcium pools, such as bones.

DENTAL SCIENTISTS STUDY THE RELATIONSHIP OF CALCIFYING MINERALS TO DENTAL DECAY

It has been a long standing concept that the susceptibility of the teeth to dental decay is determined to a large extent by certain calcifying mineral constituents of the diet. Recent data obtained from studies by dental investigators offer a better understanding of how these minerals actually influence the chemistry of the teeth and caries susceptibility. In experiments by Dr. F. J. McClure and Mr. H. G. McCann, Laboratory of Biochemistry, the ash content (calcium, phosphorus, carbon dioxide, and magnesium) of the teeth and long bones of white rats was studied following maintenance on dietary regimes widely varied in calcium, phosphorus, and magnesium. Results showed that variations in these dietary minerals appreciably affected the chemical composition of certain of the

calcifying tissues, particularly those which were undergoing mineralization during the experimental period. However, no correlation could be established with the dental caries experience of the test animals. Of additional significance was that, contrary to previous studies reported in the literature, there was no evidence that the carbon dioxide-phosphorus ratio in dental tissues was a significant factor in the incidence or severity of dental decay.

In those diets, which were supplemented with calcium and phosphorus compounds, variable cariostatic effects were noted with the calcium supplement, whereas the most significant and consistent reduction in decay was closely identified with a dibasic sodium phosphate additive. It is noteworthy that this anticaries action was not related to changes in the calcium, phosphorus, carbon dioxide, and magnesium content of the enamel or dentin.

SCIENTIST SUGGESTS PRESENCE OF NEW PROTEIN IN DENTAL ENAMEL

While previously published analyses of enamel protein have shown wide variability, they have at the same time agreed in reporting the presence of large amounts of glycine, proline, and hydroxyproline. Largely from such data it might be concluded that enamel protein belongs to the collagen class. This concept was challenged by a NIDR chemist reporting this year on studies of the protein matrix of human enamel.

Recognizing the possibility that previous samples may have been contaminated with varying amounts of dentine collagen, Dr. Karl A. Piez, Laboratory of Biochemistry, performed a series of complete amino acid analyses of enamel proteins carefully isolated from unerupted third molars. Through the use of special apparatus designed to give precise analysis, it was found that the protein was nearly all soluble in the decalcifying solution. Moreover, the soluble protein contained no hydroxyproline or hydroxylysine and only moderate amounts of glycine indicating that it was not a collagen. While its composition showed several distinctive characteristics, it was unlike any other known protein. The small insoluble residue was similar in composition to the soluble protein if the presence of about one-third collagen is assumed.

Thus, it is suggested that results from previous amino acid analyses were influenced by contamination with dentine collagen as well as losses of enamel protein owing to its solubility.

FLUORIDATION STUDIES

CLINICAL STUDIES COMPARE THE RELATION OF CARIES SUSCEPTIBILITY TO FLUORIDE CONTENT OF ENAMEL

A considerable body of evidence attests to a reduction in enamel solubility following treatment with fluoride solutions. This is believed to result, in large part, from a conversion of the hydroxyapatite of dental enamel to a more stable fluorapatite. Further important research on the cariostatic mechanism of topically applied fluoride has demonstrated large accumulations of fluoride in the extreme outer surfaces of tooth enamel. NIDR grantees reported on studies comparing the increment of fluoride in enamel and the reduction in dental caries resulting from topical fluoride applications.

The research study, consisting of both clinical trials and laboratory analyses, was undertaken by Drs. I. Sundvall-Hagland, Eastman Dental Clinic, Stockholm, Sweden; F. Brudevold, Eastman Dental Dispensary, Rochester, New York; W. D. Armstrong, University of Minnesota, Minneapolis, Minnesota; and D. E. Gardner and F. A. Smith, University of Rochester.

Clinical investigations were conducted in Stockholm, Sweden, a city where the water contains .09-.11 ppm natural fluoride. One-half of the dentitions of 107 children, averaging 2 years 8 months of age, were treated four times with a 2 per cent sodium fluoride solution. Each topical treatment lasted for four minutes and all occurred within a period of 15 days. The untreated teeth and the teeth of a group of 102 comparable children who received no fluoride treatment served as controls. The caries reductions after one, two and three years were 19, 14, and 7.5 per cent when based on a comparison of treated and untreated teeth in the same mouth; and 34, 23, and 12 per cent when teeth of untreated children were used as controls.

Laboratory analysis of the exfoliated teeth showed that topical fluoride applications caused an increase of approximately 50 ppm in the fluoride concentration of the outer portion of the enamel. There was no detectable decrease of fluoride content in the treated enamel during the 3-7 years period following treatment.

INVESTIGATORS STUDY FLUORIDE TABLETS AS CARIES CONTROL MEASURE

Although the fluoridation of public water supplies has become an accepted dental health procedure, one-third of the U. S. population does not have access to communal water supplies and may never reap the benefits of this discovery. For this group one of the alternate means of adding fluorides to the diet, which has received considerable

attention, is the use of fluoride tablets. Dr. Francis A. Arnold, Jr., Director, National Institute of Dental Research, has presented results of a long-term study of caries inhibitory effects of fluoride tablets in children. Conducted with Dr. F. J. McClure, Chief, Laboratory of Biochemistry, and Mr. Carl L. White, NIDR statistician, the study concluded that the one-a-day tablet regimen could have a beneficial effect on the teeth of growing children.

The mean numbers of DMF and def teeth of 121 children ingesting a daily fluoride tablet were compared with corresponding data previously reported for children using natural fluoride or fluoride-adjusted drinking water. Cariostatic effects of the two procedures appeared to be of generally comparable levels. The tablets, containing 2.21 mg of NaF (1.0 mg of fluoride) did not appear to cause any cosmetically significant fluorosis.

The mean fluoride content of deciduous teeth of children ingesting these tablets was found to be higher than that of children in a non-fluoride area. It also appeared that the fluoride content of teeth increased proportionately with the length of time the tablets were ingested.

Based on data gained during the test program, the investigators concluded that the tablet method, while effective, is not as practical as water fluoridation. Further, the fact that the fluoride content of the "home" water supply must be also known suggests that as a public health procedure this method could best be used in controlled programs supervised by health authorities.

LONG-TERM STUDIES CONFIRM BENEFICIAL EFFECTS OF WATER FLUORIDATION

This year the Director of NIDR participated in a special ceremony climaxing a thirteen-year independent fluoridation study in the city of Evanston, Illinois.

The community of Evanston initiated its water fluoridation program in 1947 as a result of the recommendations of Dr. H. Trendley Dean, then Director of NIDR. City officials obtained the services of Dr. J. Roy Blayney and colleagues of the University of Chicago's Zoller Memorial Dental Clinic in designing and conducting a long-term evaluation of the research program and its effects.

The subsequent survey of Evanston's school children during the thirteen year period revealed notable reductions in decayed, missing and filled teeth as compared with those of the neighboring, non-fluoridated community of Oak Park, as well as with Evanston records prior to fluoridation. These extensive studies also included such features as chemical and bacterial analyses of saliva,

water at the filtration plant and at other points in the pipeline system, tooth and bone specimens, as well as roentgenological examinations of occlusion and other oral features.

The figures reported after more than a decade of study, were based on statistics furnished by the Illinois State Department of Health, also cooperating in the survey. Results showed that children of such ages as to achieve fluoridation benefits had an average of 2.75 fewer decayed teeth than those not participating. The Evanston study adds further credience to the beneficial effects of fluoridation reported in long-term studies by the Public Health Service in Grand Rapids, Michigan; Newburgh, New York and Brantford, Ontario.

CALCIFICATION

SCIENTISTS DEVELOP TECHNIQUE FOR *IN VIVO* STUDY OF CALCIFICATION

Basic studies in the broad field of mineralization have suggested that "regulators" of calcification, or differences in the reactivity of hard or soft tissue collagens, may be factors determining which of the collagenous tissues do or do not undergo mineralization. New methods enabling *in vivo* studies of these factors have now been reported by NIDR scientists.

In collaborative studies by Drs. S. E. Mergenhagen, G. R. Martin, A. A. Rizzo, and D. B. Scott, assisted by Mr. D. N. Wright, strips of tail tendon from mature rats were implanted in the peritoneal cavities of young Sprague-Dawley rats. Samples removed at 100, 120, and 160 days were found to be hard on gross examination. Marked calcification was indicated by the typically altered calcium/hydroxyproline ratio, and by the presence in the collagen fibrils of hydroxyapatite crystals as identified by electron diffraction. Tail tendon dissected from these same animals at the times when calcified tendon implants were removed showed no evidence of mineralization. In another experiment gels of reconstituted rabbit skin collagen were also calcified when implanted (either directly or after encapsulation in dialysis bags) into the peritoneal cavities of white rabbits and rats.

The phenomenon of intraperitoneal mineralization gives rise to challenging possibilities for *in vivo* experimentation with collagen preparations which may be subjected to various treatments prior to implantation, or implanted in combination with agents thought to influence mineralization. Such living "model systems," operating under relatively well defined and controlled conditions, should provide new information on the still undetermined factors and mechanisms involved in calcification.

DENTAL SCIENTISTS MEASURE DIFFERENTIAL UPTAKE AND RETENTION OF CALCIUM AND STRONTIUM DURING CALCIFICATION

Possible hazards associated with the ingestion of Strontium^{90} from fall-out have stimulated interest in the biochemical behavior and metabolism of calcium and strontium in calcified tissues. Dental investigators at NIDR and the American Dental Association's Research Division, National Bureau of Standards, reported this year on studies of the comparative fixation of Ca and Sr by synthetic hydroxyapatite.

Strontium when taken into the body is a bone-seeker and like calcium becomes incorporated into the bones and teeth. In laboratory experiments devised by Dr. R. C. Likins, Mr. H. G. McCann, and Dr. D. B. Scott, NIDR, and Dr. A. S. Posner, NBS, trace amounts of radioactive calcium and strontium were introduced in equal quantities into solutions of calcium phosphate out of which synthetic hydroxyapatite was crystallized. By varying the precipitation conditions a number of "tagged" samples were produced with crystal sizes ranging from 20 to 4000 millimicrons.

Geiger counter measurements of the relative amounts of the two radioisotopes present showed there was a progressive discrimination against strontium deposition as the crystals increased in size. The smaller, more quickly formed crystals had approximately the same ratio of Sr^{89} to Ca^{45} as the original precipitating solution. However, experiments to determine the distribution of the respective radioactive isotopes within the crystal showed a preferential concentration of Sr at the surface. These results on synthetic materials using radiotracers may lead to a clearer understanding of the comparative uptake of calcium and strontium in the calcified tissue of the human body. Bone crystals, which resemble in size the very smallest synthetic calcium phosphate mentioned above, have already been shown to show a small but measurable discrimination against the incorporation of Sr^{89} as opposed to Ca^{45} in a series of experiments on laboratory rats.

Current studies of the differential uptake and retention of Ca and Sr in the mineral portion of the exoskeleton of certain lower animals may provide an even better understanding of the biochemical behavior of these ions.

EXPERIMENTAL PATHOLOGY

PRESERVATION AND STORAGE OF VIABLE TISSUES

The preservation and storage of organs and tissues for future use in replacement procedures have become important adjuncts to therapeutic practice. Grantees at New York University College of Dentistry

reported on studies of the effects of freezing agents and freezing rates on viable oral tissues.

The effectiveness of glycerol and ethylene glycol in preserving the viability of tooth germs subjected to various freezing temperatures was studied by Dr. R. Gerstner and other NYU investigators by explanting the tissues, after thawing, to tissue cultures, and observing the extent of cellular outgrowth and the degree of internal differentiation.

Molar and incisor tooth germs removed from 15-20 day old rat fetuses were pretreated with various concentrations of the two preservative agents. The specimens were then mounted and sealed in test tubes which were immersed in three selected freezing solutions as follows: liquid nitrogen (-198°C) for three minutes; isopentane cooled in liquid nitrogen (-120°C) for three minutes; and, dry ice in ethyl alcohol (-78°C) for 15 minutes. Following removal, the tooth germs were quick thawed in Tyrode solution and cultured in Carrel flasks. Viability results were evaluated by the extent and condition of epithelial outgrowth from the enamel organ compared with that of control, non-pretreated, non-frozen tooth germs also in culture. Differentiation within explants was examined histologically and development was compared with previously established growth norms.

Results showed that glycerol in a 30 per cent solution was the most effective pretreatment agent, and the slowest rate of freezing--that is dry ice alcohol at 78°C--was the least damaging of the freezing solutions employed. Continuation studies on the relationship of freezing temperature to freezing rate as a critical preservation factor may lead to the development of better techniques for the storage and implantation of teeth as well as other vital tissues of the body.

RELATION OF CENTRAL NERVOUS SYSTEM AND ORAL DISEASE STUDIED

For many years dental scientists have been preoccupied with studies of various local and systemic factors in attempts to discern the agents responsible for different oral conditions. Grantees at Tufts University School of Dental Medicine reported on studies of the nervous system as one of the contributing etiologic factors in diseases of the oral cavity.

The purpose of the investigation carried out by Drs. G. Quintarelli and H. H. Chauncey was to determine the effects on the oral cavity of chronic visceral stimulation of the brain stem. Experiments were performed on seventeen dogs, seven of which received traumatic stimulation by means of a blunt probe. Ten additional animals were stimulated with glass beads placed in the sella turcica thereby

producing a mild continuous pressure on the tuber cinereum, infundibulum, and mammillary bodies of the nerve system. Six animals served as controls and were operated on in the same manner as the experimental animals but received no cerebral stimulation.

All animals in the first group died by the eleventh postoperative day, showing severe hemorrhage of the gastric and intestinal mucosa and superficial erosion of the intestinal tract. No oral lesions were noted. Animals in the second group showed changes beginning on the fourth postoperative day when swelling and hemorrhaging of the oral mucosa was detected. This condition progressed until aphthous-like lesions were evident on the mucosa of the lips, cheeks and tongue. In many instances, the entire gingival area become edematous and inflamed, with progression to marginal periodotitis. Also noted was gastric bleeding and focal lesions and ulcers in the duodenum and jejunum. Control animals kept in the same environment showed no changes in either the oral cavity or intestinal tract.

Data from this study have clearly demonstrated that irritation of certain areas of the brain stem may effect basic pathologic changes in the oral cavity as well as the gastrointestinal tract. Thus, these investigations open for consideration the possible role that more subtle neurological disorders may play in the general behavior of oral diseases.

GROWTH AND DEVELOPMENT

NEW TECHNIQUE USED IN RELATING CONGENITAL DEFORMITIES TO FETAL ANOXIA

Fetal anoxia, implicated in the genesis of congenital deformities, has previously been studied in pregnant animals made hypoxic through oxygen deprivation. NIDR investigators reported this year on studies specially designed to induce anoxia directly in the fetuses of rats, thus bypassing possible complications resulting from maternal hypoxia. The effects of temporary anoxia upon fetal mortality, and upon the frequency of congenital abnormalities, including cleft palate, were studied by Miss Lee Feild, Dr. S. J. Kreshover, and Mr. J. E. Lieberman. Initially, the onset of gestation was established in 249 Sprague-Dawley rats between 2 and 3 months of age. At periods of gestation ranging from 9 to 17 days, the animals were anesthetized and both uterine horns were exposed through abdominal incision. The circulation to either the right or left uterine horn was occluded by clamping the vascular supply, and the uterus was then returned to the body cavity. (This technique provided both experimental and control fetuses within the same parent animal). After the scheduled period of circulatory arrest, ranging from 10 to 60 minutes, the clamps were removed. The uterus was again returned to body cavity, and the incision was then sutured. One day before expected term delivery, the young were extracted by cesarean section and examined.

Results showed that developmental anomalies occurred only in fetuses of the 169 rats treated between the 12th and 15th days of gestation. Twenty-six of the 507 viable experimental young in this group were deformed, whereas 2 of the 523 viable control young were deformed. The greatest frequency of deformities (17.6 per cent) occurred after a 45 minute vascular occlusion on the 15th day. There were 13 instances of palatal clefts, 8 of which occurred in the progeny subjected to anoxia on the 14th day. In addition, there were 18 experimental young with limb defects or other abnormalities, 5 of which also had cleft palates.

Data on fetus mortality showed that a high incidence of death was also related to circulatory interference, particularly after occlusion on the 13th gestational day, and after the longer periods of circulatory arrest.

BONE DEVELOPMENT IN CHICK EMBRYOS ALTERED BY TETRACYCLINE

Frequently used in clinical practice as a broad-spectrum antibiotic, tetracycline is a compound of wide interest to dentistry, both as a prophylactic measure in oral surgery and because of its potential as a caries inhibitor. Grantees at the New York University College of Dentistry, have now reported on studies to determine the possible effects of tetracycline on skeletal formation. In studies designed by Dr. Gerrit Bevelander and associates to test the effects of tetracycline on growing bone, the compound was injected, in amounts ranging from 0.1 to 2.5 mg., into the yolk-sac of eight-day old chick embryos. It was observed that the 0.5 dose was at first distributed uniformly throughout the embryo. Twenty-four hours later, however, it appeared exclusively in the calcified portions of the skeleton and was identified by its characteristic fluorescence in the presence of ultra-violet light. The 2.5 mg. injections resulted in a pronounced retardation in over-all growth of the 10, 12, 14, and 16 day old chicks. Microscopic examination of the femurs of embryos treated with this relatively large dose showed the following abnormal characteristics: a marked bending, a reduction in the number of trabeculae, and the degree of mineralization was less than half that observed in normal bones. Additional studies of this family of antibiotics are contemplated in light of their frequent use in dental practice on children during the growth and development period.

EPIDEMIOLOGY

EPIDEMIOLOGIC STUDY OF ORAL DISEASE IN RELATION TO NUTRITION

Surveys to identify patterns of association between nutrition and oral disease have been completed in Alaska, Ethiopia, Peru, Ecuador, and Vietnam. Results of these studies, conducted in

cooperation with the Interdepartmental Committee on Nutrition for National Defense, were published in summary form this year. In general, epidemiological studies of foreign and domestic population groups have failed to establish a consistent association of dental caries with a deficiency of any known nutrients. For example, both high and low levels of caries experience in Alaskan Eskimos were associated with high intake of protein, moderate intake of fat, low consumption of carbohydrate, and low plasma level of Vitamin A. On the other hand equally low, as well as lower caries prevalence levels were seen in Ethiopians subsisting on a comparatively low-protein, high-carbohydrate cereal diet, high in thiamine; and in Vietnamese on a highly milled rice diet yielding only marginal amounts of thiamine and riboflavin. Also unexplained were the large proportions of individuals in each of the groups that were totally free of dental decay.

Where caries levels in these populations were generally low by U. S. standards, the prevalence and severity of periodontal disease were correspondingly high. For example, in Ethiopia, Ecuador, and Vietnam, the levels of periodontal disease were much above any so far recorded for the continental United States. Deviations from this pattern of prevalence were noticed among primitive groups of Alaskan Eskimos and the Ecuadorian Indians, where a reduction in periodontal disease was related to higher thiamine excretions and lower levels of plasma Vitamin A. Elsewhere in Alaska, and generally in Peru, periodontal disease levels were comparable with those seen in the United States among people of low socioeconomic status.

EPIDEMIOLOGY STUDIES INCLUDE SEVENTH DAY ADVENTIST CHILDREN

Previously reported epidemiologic studies of dental disease in Seventh Day Adventist children have indicated a significantly lower evidence of dental caries than noted in other population groups. NIDR investigators have recently completed studies which assessed the caries experience of Adventist and non-Adventist children under more comparable environmental conditions.

In studies conducted by Dr. C. J. Donnelly, Epidemiology and Biometry Branch, a total of 290 Adventist children and 1438 non-Adventist children, all lifetime residents of Prince Georges and Montgomery Counties in Maryland, were examined for dental caries. The children were 6 through 14 years of age and all had received fluoride adjusted drinking water since 1952. Sex and age distribution in the two groups, as well as general environmental conditions, were quite comparable.

Results, based on examination for decayed, missing, and filled teeth, showed that there was little difference between the 6 to 9 year old children in the two groups. These age categories

benefited most from the 7 years of fluoridation. However, in the 10 through 14 year categories, there was a 40 per cent discrepancy in the mean number of DMF teeth in favor of the Adventist children.

Data from this study suggest further investigation of a possible caries inhibitory factor in the Adventist way of life. Recognizing that fluoridation may have rendered imperceptible such an inhibitory factor, particularly in the younger children, it is planned to continue with parallel studies in areas where caries attack is not influenced by the ingestion of water-borne fluoride.

PROGRAM HIGHLIGHTS

CONFERENCE OF GRADUATE TRAINING PROGRAM DIRECTORS SPONSORED BY NIDR

A serious shortage of dental teachers and researchers in the decade ahead has now been confirmed in studies by the American Dental Association and Special Consultants to the Senate Appropriations Committee. To consider this problem, the National Institute of Dental Research called together the Directors of established Graduate Training Centers in July 1960, for discussion of program objectives and expanded efforts in the basic sciences and clinical dentistry.

Speaking before Program Directors and members of the National Advisory Dental Research Council convened at Western Reserve University, Dr. F. A. Arnold, Jr., Director, NIDR, pointed out that in the next ten years, a minimum of 4700 new faculty members will be needed in the basic and clinical science departments of our Nation's dental schools, where today only one-fifth of the faculty members teach full time. Research and teaching manpower needs will mount at a rate of 500 trained individuals a year, Dr. Arnold predicted, and all areas of dental activity--particularly teaching and research--will expand at less than an optimum rate if this central problem remains unresolved. As estimated by the Council on Dental Education, ADA, the cost of training manpower commensurate with needs, under the Federal grant and fellowship programs, is expected to total 12½ million dollars annually based on today's dollar value.

Highlighting the two day program were presentations by prominent figures in the fields of dental education and research. Subjects included: curriculums for graduate clinical and basic science training, part-time vs. full-time training, recruitment methods and resources, and the influence of graduate training programs on dental education. New concepts in curriculum design and recruitment procedures were discussed in light of the demonstrated preference of college graduates for Federal subsidized Ph.D. training, and

recent recommendations of Federal assistance for undergraduate dental training for those students interested in research and teaching careers.

AMERICAN DENTAL ASSOCIATION TO SPONSOR GENETICS SYMPOSIUM AT NIH

Hereditary factors have long been associated with dental related problems such as defective calcification, malocclusion, cleft lip and palate, periodontal disease and caries susceptibility. Currently emphasizing the difficulty of clarifying the influence of heredity is the evidence that many types of environmental agents can be shown experimentally to produce anomalies identical to those produced by defective genes. To further the study of hereditary factors involving the entire organism and particularly their role in oral malformations and disease, the Council on Dental Research of the American Dental Association in cooperation with the National Institute of Dental Research announced in June, 1960 that it will conduct a grant-supported symposium at the National Institutes of Health early in 1961. The program titled "Symposium on Genetics Related to Oral Health," will feature prominent geneticists, and dental and medical investigators from this country and abroad. Its purpose will be to exchange information on present and proposed studies in genetics, thus stimulating greater progress in the field; acquaint the dental profession with recent developments in genetics; and more clearly define the following areas: (a) recent developments in medical genetics, (b) hereditable diseases of dental interest, and (c) genetic methods applicable to the investigation of intrinsic dental disorders.

Research papers presented at the three-day session will be published in book form to provide dental and medical investigators with comprehensive source material in the expanding field of genetics related to dentistry.

NEW DENTAL JOURNAL IS PUBLISHED

The first issue of Dental Progress, a new scientific journal that seeks to bridge the gap between the researcher and the clinician, was published in October 1960. A National Institute of Dental Research grant assisted in the establishment of the new medium designed to complement the existing, more specialized journals in the field of dentistry.

With an almost explosive increase in the number of research grants beginning in 1957 (fivefold increase over 1956), the National Advisory Dental Research Council was not unmindful of the impact this unprecedented support would have in ensuing years on the existing family of professional dental journals. Therefore, in 1957, the Council appointed a Publication Committee to study the

broad problem of communication within the profession, with particular emphasis on ways and means to accelerate the prompt reporting of new research data.

In June 1959, the Advisory Council, acting on recommendations of its Publication Committee, and in consideration of the particular need for a new medium of broad interest to complement the more specialized journals, recommended the award of a grant to the University of Chicago Press to assist in the establishment and publication of Dental Progress. This grant was approved by the Surgeon General in July 1959.

HIGHLIGHTS OF PROGRESS

IN

MENTAL HEALTH RESEARCH

1960

Items of Interest on Program Developments and Research Studies

Conducted and Supported by the National Institute of Mental

Health

The problem of mental illness has plagued and mystified mankind since earliest times. Yet probably in no area of medicine and science has progress toward basic understanding of causative factors been so arduously slow or painfully achieved. It was more than 2,300 years after the Hippocratic physicians described with remarkable perception some of the classic symptoms of mental syndromes that a comprehensive classification of forms of mental illness was developed. Shackled by superstition, ignorance and fear, men for centuries despaired of solutions to the enigmas of abnormal behavior. The first great advance in understanding and treatment came in the late 18th century, with the gradual development of the concept of mental illness as a definite sickness based on organic brain pathology. Only as recently as the turn of the twentieth century was the role of psychological factors discovered and formulated.

Today, research in the field of mental health and mental illness is extremely diverse. Biological, psychological, and sociological studies are being conducted in a broad multidisciplinary approach to the numerous difficult and unsolved problems. Many of these studies bear an obvious relation to the painstaking task of picking away at the tangled threads of the etiology of mental illness. Other studies are more or less directly aimed at improved methods of treatment and rehabilitation of the mentally ill. But there are many investigations in the field of mental health which, on the surface, have no apparent link to the attack on mental illness. These are the investigations at the more basic level, which attempt eventually to throw new light on the disordered mind. To understand what happens to the disordered mind, it is essential to understand the workings of the human mind under normal conditions, and to understand the human mind, it is essential to understand the many complex interrelationships of the mind with its environment--both the interior physiological environment of the

human body and the exterior psychological and sociological environment of the interpersonal world and society. For this reason, much of the research in the field of mental health is basic research, both at the biological and psychological levels, and occasionally several steps removed from the core problems of emotional disorders. These studies seek to find answers from disparate sources which will fit together to make a tool with which to unlock some of the recondite mysteries of mental illness.

This report presents selected findings of research conducted during the past year in the Institute's own laboratories and in many nongovernmental research centers throughout the country which have received support from the Institute.

PROGRESS IN TREATMENT

MENTAL HOSPITAL POPULATIONS DECLINE

New therapies, including an ever-expanding list of psychopharmacological agents, new techniques both in and out of the mental hospital, and the development of new mental health programs and treatment facilities are gradually reducing the number who must be institutionalized in public mental hospitals.

For the past 5 years, resident mental hospital populations have continued to decline. Data from the Institute's Biometrics Branch indicate that the 535,332 patients resident in the 281 public mental hospitals at end of fiscal year 1960 represented a decrease of 7,389 from the corresponding number at end of fiscal year 1959. This decrease of 1.4 percent compares with a decrease of 0.4 percent between 1958 and 1959, 0.7 percent between 1957 and 1958, 0.5 percent between 1956 and 1957, and 1.3 percent between 1955 and 1956. These figures incorporate the effects not only of the number of releases but also of the number dying and the total admissions occurring within the year. Data indicate that total admissions increased 4.3 percent, 4.8 percent, 7.7 percent, 6.5 percent, and 5.3 percent in 1956, 1957, 1958, 1959, and 1960, respectively, over the previous year; the corresponding percent increases in the number of net releases was 14.6 percent, 3.8 percent, 7.7 percent, 8.5 percent, and 9.6 percent; and the corresponding changes in the number of deaths was 8.7 percent, -2.9 percent, 9.5 percent, -3.3 percent, and 0.3 percent.

IMPROVING THE HOSPITAL ENVIRONMENT

Much attention is being given to the problem of how to make the mental hospital environment more therapeutic. Inservice training programs are improving staff skills and are bringing the benefits of new experiments in hospital administration to more and more mental patients.

For example, an exchange training program among 13 Southern States during the past 2 years has enabled personnel in mental hospitals to observe at first hand new techniques of hospital administration and patient care. The program, conducted by the mental health unit of the Board of Control for Southern Regional Education, was made possible by an Institute grant. Under the program, psychiatrists, psychologists, social workers, nurses, physicians, and other hospital and training school personnel have been given up to 4 weeks inservice training at other institutions.

LAWS ON MENTAL ILLNESS

Much of the current legislation governing commitment and discharge of mental patients interferes with prompt and effective treatment and with their speedy rehabilitation and reintegration into community life. Measures depriving the mentally ill of their civil and other rights represent a confusing patchwork of laws which are a heritage from the past. If modern methods of therapy and rehabilitation are to be effective, the legal hobbles which tend to make invalids of the mentally disabled and prevent their rehabilitation, must be removed.

A major step toward the revamping of legislation dealing with the mentally ill has been taken by the American Bar Foundation, which has just completed a 3-year survey, a major portion of which is a study of all major civil and criminal laws and commitment procedures related to mental illness. The report of this study, to be published under the title of The Mentally Disabled and the Law, compares statutes, regulations, and court cases dealing with commitment and discharge of mental patients in the various States. Other aspects discussed include determination of the need for protective procedures at discharge, and protection of personal and property rights of committed mentally ill persons under present laws. Legislation pertaining to marriage, divorce, eugenic sterilization, incompetency, guardianship, restoration, sexual psychopathy, and mental disability as related to criminal law also receives attention. A study of voluntary commitments is included.

Building on the groundwork provided by this comprehensive survey, the American Bar Foundation is now conducting a follow-up study, with the aid of an Institute grant, designed to develop

model legislation governing care and hospitalization of the mentally ill. Evaluation of existing practices will prepare the way for draft legislation, which can be adopted by the individual States to insure proper treatment of the mentally ill, while at the same affording adequate protection of their civil rights.

POLICE TRAINING IN HANDLING THE MENTALLY ILL

Because the police often are called upon to deal with the mentally ill, particularly in emergency situations where the ill person may be highly excited and may require immediate hospitalization, a number of communities have instituted special training sessions for the police on this subject. A manual for police officers on How to Recognize and Handle Abnormal People, prepared a few years ago by Dr. Loyd W. Rowland of the Louisiana Association for Mental Health and Dr. Robert A. Matthews, has been used very successfully in such training programs.

During the past year, with support from the Institute, Dr. Rowland and the Louisiana Association, in cooperation with the New Orleans Police Department, produced a new training film for the police entitled Booked for Safekeeping. This 33 minute, 16mm. sound film provides basic information needed by any type of law enforcement officer in handling disturbed mental cases, and will assist him in understanding patterns of abnormal behavior. The film depicts average and typical conditions under which the police normally must operate. It contains information useful also to employees of penal institutions and courts. Provisions have been made for widespread utilization of the film by local police departments, law enforcement agencies and associations, and training centers for police officers throughout the country.

POST-HOSPITAL EXPERIENCE OF MENTAL PATIENTS

With increasing numbers of patients achieving remission and returning to the community, considerable research activities are directed at developing techniques not only for preventing relapses but also toward improving the level of the ex-patient's personal functioning and adjustment. An intensive study of post-hospital experience has been conducted, with Institute support, over a period of years at the Harvard School of Public Health, in an effort to determine what actually happens to patients when they leave the mental hospital. Findings from this study, compiled and published this year by the Hogg Foundation for Mental Health, underscore the importance of evaluating the community milieu.

The role the former patient may be expected to assume in the home, for example, is a critical determining factor. The prognosis for returning husbands is not so good as that for sons.

Hospitals releasing incompletely recovered patients to wives are likely to have to readmit them, and alternative community settings for such men are in many instances indicated. The study suggests that a higher proportion of sons now hospitalized might be returned to their families.

Conclusions of the study also suggest a need for appraisal of the practice of releasing patients who are less than well on the assumption that treatment will continue after hospitalization. Investigators found that many patients returned to the community remain there, "preserved like flies in amber," living as it were in one-person chronic wards where they are given custodial care by family members. In such situations these patients are insulated from treatment agencies and other community services. It was found that often the family setting in which the returned patient was tolerated was not therapeutic and rehabilitative, although permissiveness and acceptance were provided. Low-level performance and bizarre behavior on the part of the former mental patient were found to occur most often in those families with low expectations regarding post-hospital behavior. A family with high expectations at the time the patient returned, which remained high, was most conducive to motivating the patient to produce a higher level of performance.

The results of the study underscore the urgent need for further mobilization of the interest and efforts of both hospital and community to ameliorate problems in this area.
(NIMH grantee Dr. Ozzie G. Simmons, Harvard University. Reported at meeting of the Texas Association for Mental Health.)

DRUG THERAPY FOR RELEASED PATIENTS

Follow-up studies of released mental patients, conducted under grants awarded by the Institute, have shown that psychoactive drugs, which have proved so effective in hospital treatment of mental illness, can be used successfully in preventing the rehospitalization of chronic schizophrenic patients and in making it possible for them to live at home and hold regular employment. The prolonged treatment procedure is safe, given adequate safeguards, chief among which is proper skilled medical supervision. The drugs used do not appear to bring about adverse side effects nor to impede the patient at his work or learning.

One pilot study was conducted last year by the Research Unit of the Manhattan Aftercare Clinic in New York. A special Day Hospital facility was set up for the treatment of patients who after release suffered a recurrence of psychotic symptoms. During the year, 26 patients who suffered such relapses were given intensive drug therapy at the Day Hospital under the supervision of psychiatrists, social workers, and nurses. The

required drug dosage was adjusted daily. The patients were given occupational therapy. When family problems were involved, the social worker counseled and helped. In all cases, the psychotic symptoms were brought under control within 6 weeks or less, and the patients were able to return to their duties. Absence for such a limited period did not result in job loss.
(NIMH grantee Dr. Elsa Kris, Manhattan Aftercare Clinic, New York City. Reported at Conference on Drugs and Community Care, Washington, D. C.)

In a 4-year follow-up study of 330 patients treated with drugs after discharge from the Delaware State Hospital, it was found that only 13.8 percent of those receiving the drug suffered relapses which required rehospitalization, while 47.2 percent of those not kept on treatment had to return to the hospital.
(NIMH grantees Fritz A. Freyhan, M. D., and Joan Merkel, R.N., Delaware State Hospital. Reported at Conference on Patterns of Transition from Hospital to Community, Boston.)

Since psychological and social factors enter into the shifting mental status of a patient under drug treatment, several studies have been designed to separate out the effect brought about solely by medication. Such a study was conducted at the psychopharmacological research unit of the State University of New York. Medication was given, along with brief psychiatric interviews, to 173 schizophrenic outpatients in the form of capsules containing either placebo, promazine hydrochloride, or chlorpromazine hydrochloride. During the course of treatment, 29 patients (16.8 percent) were hospitalized for mental illness. An analysis of the research record showed that 28.6 percent of the placebo patients were hospitalized while 18.2 percent and 4.8 percent of the promazine and chlorpromazine groups, respectively, were hospitalized.
(NIMH grantee Dr. David Englehart, Kings County Hospital, Brooklyn, New York. Reported at Conference on Patterns of Transition from Hospital to Community, Boston.)

AVOIDING HOSPITALIZATION

New methods are being tried out in an attempt to obviate the necessity of hospitalizing mentally ill persons when they can be treated on an outpatient basis.

In one community where it has been tried as an experiment, emergency psychiatric treatment in the home, for patients for whom admission to the State hospital had been requested, has proven to be an effective alternative to hospitalization. When the emergency arises, a team composed of psychiatrist, social worker, and nurse visits the family and works with it as a group and as individuals. Diagnosis and treatment are given by

the clinical team when it is felt the patient can remain outside the hospital. During the first 2 years of its operation, the psychiatric home team saw 75 cases for a total of 800 home visits. Eight families were accepted for long-term treatment. The remaining cases were given psychiatric evaluation, brief therapy, and/or referral to other agencies or private physicians. The project was conducted under the direction of Dr. Walter E. Barton of the Boston State Hospital with the aid of an Institute grant.

Another experimental program conducted by the Department of Psychiatry, University of Texas, has shown the effectiveness of brief intensive multiple-impact psychotherapy in permitting outpatient treatment of severely disturbed adolescents. The adolescents who ordinarily would have required long-term psychotherapy or institutional care, have been treated in concentrated 2-day therapy sessions at 6- to 8-week intervals for a period of 2 years. Under the program, the adolescent and his entire family come to the clinic for the 2-day period. Of the 50 adolescents and families followed in the program for a 2-year period, all but 7 showed definite improvements, and 38 adolescents were still living with their families who had effectively mobilized their forces to cope with the youngsters' problems. (NIMH grantee Dr. Harold A. Goolishian, University of Texas Medical Branch. Reported in Social Work.)

Outpatient mental health clinics for years have helped large numbers of people with a wide range of emotional disorders. This year, a report of a survey of ambulatory patients in mental health clinics, the first of its kind so far conducted, reveals that an estimated 379,000 individuals in the United States, including 197,000 children and 182,000 adults, were clinic patients during 1955. One-third of the child patients with diagnosed disorders had transient situational personality disorders. Personality disorders and mental deficiency were frequently diagnosed. Adult patients were usually reported as having a personality or psychoneurotic disorder, although psychotic disorders were not infrequent. Relatively short-term service was provided for many patients. Median number of interviews for children was four; for adults, three. (Anita K. Bahn and Vivian B. Norman, NIMH. Reported in Public Health Reports.)

BIOLOGICAL STUDIES

Biological research in the field of mental illness covers a wide range of studies, including investigations of possible biochemical and metabolic defects, of neurophysiological irregularities, and of basic neuroanatomical structures.

Many cogent arguments point to the highly differentiated metabolism of the brain as likely to harbor important chemical mediators of normal and abnormal behavior. Recent years have witnessed the dramatic discovery of a number of neurochemical substances, the biogenic amines, which appear to play an important part in brain activity and the transmission of nerve impulses. Among them are tryptamine, serotonin, epinephrine (adrenalin), norepinephrine, histamine, and gamma amino-butyric acid. In the process of brain and body function, these biogenic amines are acted upon by enzyme systems which synthesize and destroy them. Much has been learned about the pathways through which these metabolic processes take place. As knowledge of their synthesis, degradation, and distribution is gained, it lays the foundation for comparative studies that may reveal abnormalities that provide clues to mental illness.

EPINEPHRINE AND SCHIZOPHRENIA

A major theory formulated in recent years to account for schizophrenia in biological terms has been based on a postulated disorder in the metabolism of circulating epinephrine, with the production of certain abnormal derivatives. This theory was developed at a time when it was possible to account for only a small percentage of the epinephrine released in the body, and only that which was excreted unchanged in the urine. There was no means of measuring the epinephrine altered by body metabolism. To test the hypothesis that a defect in the metabolism of epinephrine might account for the major symptoms of schizophrenia, it was necessary first to ascertain the normal metabolic pathway. In a series of studies continuing over the past several years, investigators at the Institute's Laboratory of Clinical Science previously had succeeded in determining the fate of practically all epinephrine administered and also that presumably produced in the body in the form of two major metabolites, metanephrine and 3-methoxy-4-hydroxymandelic acid, accounting for approximately 80 percent, and 4 minor metabolites including unchanged epinephrine, bringing the total which can now be accounted for to 98 percent in animals and normal man.
(Dr. Julius Axelrod, NIMH. Reported in Physiological Review.)

Based on this research, a definitive study of the metabolism of circulating epinephrine in a group of schizophrenic patients was completed this past year. The metabolites in this population were found to be qualitatively identical with that in the normal population, with no evidence for the presence of abnormal metabolites. Only one compound, 3-methoxy-4-hydroxyphenylglycol,

was excreted to an extent significantly different from that in normal volunteers (8.9 percent as opposed to 7.1 percent). Conclusions argue against the likelihood that a defect in metabolism of circulating epinephrine accompanies schizophrenia. (Drs. Elwood H. LaBrosse, Jay D. Mann, and Seymour S. Kety, NIMH. Reported in the Journal of Psychiatric Research.)

METABOLISM AND INACTIVATION OF CATECHOLAMINES

Institute investigators have delineated the metabolic features of the metabolism of norepinephrine and have elucidated a number of areas in which this metabolism differs quantitatively from that of epinephrine. Even more significant have been findings on the mechanisms of the binding and inactivation of norepinephrine at or near its site of action. In animal studies using injections of tritium-labeled norepinephrine of high specific activity, the researchers have demonstrated a rapid uptake of this hormone by certain tissues of the body which are richly innervated by the sympathetic nervous system. Denervation of such structures results in a loss in the ability to bind norepinephrine, and a number of drugs including reserpine, chlorpromazine, amphetamine, and cocaine have the ability to prevent this binding or to cause the release of bound norepinephrine from these sites. The tranquilizers, reserpine and chlorpromazine, although similar in clinical effects, have very few chemical effects in common. The similar action observed in connection with norepinephrine may provide insights into the mechanism of their psychoactive action.
(Dr. Julius Axelrod, NIMH. Reported to the Ciba Symposium on Adrenergic Mechanisms, London. Dr. L. G. Whitby, Rockefeller Travelling Fellow, Dr. G. Hertting, Visiting Scientist, and Dr. Julius Axelrod, NIMH. Reported in Nature.)

OTHER CLUES TO SCHIZOPHRENIA

A significant positive finding suggests the presence in plasma of schizophrenic patients of a factor that alters the carbohydrate metabolism of red cells. During the past year, Institute facilities were made available to a research scientist to apply this test to schizophrenic patients and normal volunteers on the wards. Appropriate analyses were carried out in the Institute's Laboratory of Clinical Science. On the basis of blood samples submitted from 12 schizophrenic patients and 8 normal volunteers, and carefully coded to prevent their identity from being known, it was possible on the basis of his findings to predict accurately in a highly significant number the presence or absence of schizophrenia.
(NIMH grantees Drs. Charles Frohman and Jacques Gottlieb, Lafayette Clinic and Wayne State University College of Medicine, Detroit. Reported in the American Journal of Psychiatry.)

ROLE OF THE BIOGENIC AMINES

The biogenic amines, which have noticeable pharmacological effects elsewhere in the body, are now known to be differentially localized in various parts of the brain. Although studies in the blood or urine may serve to detect general abnormalities in the metabolism of these agents, if they occur, they are not yet sufficiently sensitive to elucidate their specialized metabolism in the brain. An important new technique has now been developed by an Institute investigator for more precise measurements of metabolic processes. The technique, based on double-labeling, employs the use of two different isotopes (radioactive hydrogen and carbon) and may be adopted to yield information on the brain metabolism of biogenic amines from studies of blood or urine.
(Dr. I. J. Kopin, NIMH. Reported in Science.)

Another approach employs the administration of certain known precursors of certain biogenic amines in substantial amounts in the diet or bloodstream, in conjunction with drugs which inhibit monoamine oxidase. This would be expected to retard the destruction of the amines and increase their concentration in the brain. Careful behavioral and psychiatric observations of patients under such conditions may serve to elicit mental or behavioral changes which may be characteristic of alterations in specific amine levels. During the past year, such tests have been made on a number of amino acids including tryptophan, phenylalanine, histadine, glutamine, tyrosine, glycine, and methionine, and two more immediate precursors of norepinephrine and serotonin. When approximately ten times the normal dietary content of these amino acids was administered in successive studies, each for a period of one week, two of them were associated with significant behavioral changes in a segment of the patients. Methionine was associated with increased activity and alertness, and an accentuation of some specific schizophrenic symptoms, which was followed by what appeared to be a moderate quiescence of certain symptoms in a number of the patients. Tryptophan, especially in conjunction with the monoamine oxidase inhibitors, produced a relaxation of inhibition and a freeing of association where these had been abnormally marked in several patients. These findings are being further evaluated in order to elucidate their significance to the nature of schizophrenia and the relationship of the biogenic amines to behavior.
(Drs. William Pollin, Philippe V. Cardon, and Seymour S. Kety, NIMH. Science, in press.)

EFFECTS OF RESERPINE IN NERVE CELLS

Institute investigators, studying the distribution of epinephrine and norepinephrine in the brain nerve cell, have found that they can be separated into two broad fractions: the first present in the cell sap, the second

concentrated in granular matter. According to current concepts, the fraction in the cell sap is biologically active; the other is thought to serve as a reserve supply. The effects of the tranquilizer, reserpine, and a number of other drugs have been examined alone, and in combination with each other.

Findings suggest a reappraisal of previous theories on the mode of action of reserpine. The results further substantiate other recent research by Institute investigators indicating that the enzyme, catechol-O-methyl-transferase, takes part in the metabolism of norepinephrine in the brain.
(Dr. H. Weil-Malherbe, Visiting Scientist. Reported in the Journal of Neurochemistry and the Journal of Pharmacology and Experimental Therapeutics.)

MEASURING VMA EXCRETION

One of the important accompaniments of human stress is the release from the adrenal gland and the sympathetic nervous system of the important hormones epinephrine and norepinephrine (also known as the catecholamines). A precise method for measuring the amount of these agents released, and correlation with measurements of stress in health and disease, has long been sought. Techniques available for deriving such information have depended on analysis of the hormones per se in the urine. These have been unsatisfactory, since only a small percentage of the epinephrine and norepinephrine released is excreted unchanged. However, the recent identification by Institute investigators of the metabolic products of these hormones has made it possible to estimate more closely, from the residue appearing in urine, the total amount secreted. A new technique has now been developed for the determination of 3-methoxy-4-hydroxymandelic acid (VMA) in the urine of man. Since this compound in urine accounts for approximately 40 percent of injected and presumably endogenously produced epinephrine, it is a much closer indicator of catecholamine secretion in various states. The method has been applied in a number of clinical cases and it has been found, for example, that there is a high excretion rate in patients with involutional depression. A marked increase has also been observed to result from certain drugs or procedures such as reserpine and electroshock therapy.
(Mrs. Virginia Weise, Dr. Roger McDonald and Dr. Elwood H. LaBrosse, NIMH. Clinica Chimica Acta, in press.)

PRECISE ASSESSMENTS OF ENDOCRINE FUNCTION

With the development of a comprehensive battery of hormone measurements at the Institute, precise assessments of adrenal-thyroid function can now be made, and minute fluctuations in their levels can be related to fluctuations

in human behavior. The techniques enlarge the possibilities for clarifying the relationships between endocrine function and psychologic stress, which has not been fully understood. Findings, for example, suggest a change in the old concept that adrenal hormones are elevated only as an emergency measure. Research indicates that adrenal hormones sensitively reflect hour-to-hour and day-to-day experiences, and are geared to the stresses of ordinary living.

In a series of psychoendocrinological studies conducted during the past year at the Institute with normal volunteers, involving the relations between endocrine function, normal variation in emotional states, and a variety of psychological stresses both induced and occurring spontaneously, it was found that adaptation to a new environment was a potent stimulus for adrenal activity, as manifested in steroid excretion in urine. Repeated studies over several months have shown that individuals are fairly consistent in adrenocortical function. Some regularly respond to the stresses of everyday living with substantial corticosteroid output. Others under the same circumstances show relatively light response. Mildly pleasant absorptions, such as watching a "bland" nature film, lowered hydrocortisone concentrations in the blood; gripping and arousing films maintained relatively high levels.

Investigation of endocrine correlates of severe psychological stress is also underway at the Institute, in an effort to determine whether other hormones are affected under conditions already shown to be associated with elevated blood levels and urinary excretion of hydrocortisone, epinephrine, and norepinephrine. Output is being measured for aldosterone, androgens, and estrogens, via urinary excretion; and, as an index of thyroid function, blood levels of butanol-extractable iodine are being studied. Findings strongly suggest that substantial changes occur in output of these hormones in distressed states.
(Dr. David Hamburg, NIMH. Reported to the Association for Research in Nervous and Mental Diseases.)

SYNTHESIS OF MELATONIN

The pineal gland, a small structure directly in the center of the brain, has challenged the imagination of scientists for centuries. To date its function is unknown. Recently scientists elsewhere have isolated an agent, melatonin, which appears to be highly localized in the pineal gland, apparently the only structure in the brain with the ability to synthesize it. Now investigators in the Institute's Laboratory of Clinical Science, in cooperation with scientists in the National Heart Institute, have demonstrated the mechanism of the synthesis of melatonin by enzymes found in the pineal gland. These enzymes affect the

acetylation and methoxylation of serotonin with the production of melatonin. After being formed, melatonin is distributed to all tissues including the brain, and its degradation is mainly by 6-hydroxylation, with the excretion of the hydroxylated compound in the urine and bile. Although the function of melatonin in the brain is at present unknown, in lower animals it is known to be the most powerful melanocyte-contracting substance (lightening agent) known. The relationship between these cells, which control pigmentation in lower animals, and brain function is obscure. (Dr. Julius Axelrod, NIMH, and Dr. Herbert Weissbach, NHI. Reported in Science.) (Drs. Irvin Kopin and Julius Axelrod, NIMH; Dr. C. M. B. Pare, Visiting Scientist, NIMH; and Dr. Herbert Weissbach, NHI. Reported in Biochimica Biophysica Acta.)

ANIMAL DISEASE THAT MIMICS MULTIPLE SCLEROSIS

Experimental allergic encephalomyelitis, a disorder that can be induced in guinea pigs by intracutaneous injection of brain tissue, offers a useful model for the investigation of multiple sclerosis and other demyelinative or degenerative diseases. An Institute investigator has now succeeded in purifying a water-soluble brain protein from which significant immunologic results have been obtained, suggesting the possibility that the disease may be susceptible to pretreatment as well as suppression after the disease has been induced.

Although all routine immunologic tests on the active protein have been negative, carefully controlled skin testing indicates that intracutaneous injections of aqueous solutions of the protein provide means of suppressing the disease. Other inactive proteins isolated from the brain do not afford this protection. The source of the protein has been traced to the wall of the nerve cell (myelin). The findings open interesting horizons with regard to therapy and prevention of related neurological diseases in humans.
(Dr. Marian Kies, NIMH, in collaboration with NINDB grantee Dr. Ellsworth C. Alvord, Jr., University of Washington Medical School, Seattle. Reported at meetings of the Federation of American Societies for Experimental Biology and the American Association of Neuropathologists.)

ISOLATION STRESS AND BRAIN CHEMISTRY

The fact that sensory deprivation causes deviant behavior in man and laboratory animals has long been known. Now investigators have learned that isolation, a form of sensory deprivation, not only produces abnormal behavior in mice but also causes noticeable changes in brain chemistry. Studies on isolated mice have disclosed a significant lowering in phospholipid metabolism in the brain. In view of the fact that chlorpromazine, a tranquilizing

drug known to have beneficial behavioral effects in cases of mental illness, is known to stimulate phospholipid metabolism, the findings provide further leads for investigation of the yet unknown relationship between phospholipid metabolism and abnormal behavior.
(NIMH grantees Drs. Arthur W. Wase and Jens Christensen, Hahnemann Medical College, Philadelphia. Reported in AMA Archives of General Psychiatry.)

BRAIN DAMAGE AND ANIMAL BEHAVIOR

The difficulties in behavior suffered by monkeys when the frontal lobes of the brain are damaged have been traditionally regarded as due to loss of memory. Evidence now suggests that the behavior reaction that ensues may be due to abnormally strong tendencies to repeat actions over and over again (perseveration). It has also been possible to distinguish perseveration from loss of inhibition, and to show that animals with orbital frontal lobe lesions perform more poorly on problems involving inhibition, while those with lateral frontal lobe lesions perform more poorly on problems that involve perseveration. The findings may provide better understanding of the function of the frontal lobe in man, in whom perseveration and inhibition loss is frequently evident following frontal lobe injury, whereas no evidence of memory loss has been found.
(Dr. S. Brutkowski, Visiting Scientist, and Drs. M. Mishkin and H. E. Rosvold, NIMH. Reported to the Eighth Congress of the Polish Physiological Association, Poznan, Poland.)

BEHAVIORAL EFFECTS OF HORMONE IMPLANTATION IN THE BRAIN

A number of hormones are known strikingly to affect behavior, though their mode of action on the brain is far from clear. In an attempt to analyze the effect of sex hormones on cerebral mechanisms, a guest scientist has developed a method by which a small amount of solid hormone (stilbestrol n. di butyrate) can be implanted directly into the brain of cats. It was shown that implantation of hormone into the posterior hypothalamus markedly alters sexual behavior of such animals. The effect on behavior is specific for the hormone and the site of implantation. Similar implants elsewhere in the central nervous system (or blank implants in the hypothalamus) exerted little or no specific behavioral effect. The distribution of the hormone locally in the brain was studied by the use of radioactive (C^{14}-labeled) hormone. These studies suggest that a small and highly localized area of the brain immediately adjacent to the site of the implant

is subjected to a prolonged, steep concentration gradient of hormone; and also suggests that some neurones in the hypothalamus are selectively sensitive to the action of estrogenic hormone. (Dr. R. P. Michael of London, Rockefeller Travelling Fellow and Guest Scientist at NIMH. Reported to the Fourth International Neurochemical Symposium on the Regional Chemistry, Physiology and Pharmacology of the Nervous System, Varenna, Italy.)

NEW KNOWLEDGE OF NEURAL FUNCTION

One of the major mysteries in physiology is how the combination of highly excitable neuron systems in the central nervous system are maintained in orderly activity, and why such disordered discharges as occur, for example, in epilepsy are comparatively infrequent. A series of studies has been conducted in an effort to determine how discharges of these excitable systems are controlled, and what happens when control is impaired and parts of the brain convulse. The hippocampus was selected as site of study because of its exceptional susceptibility to convulsive activity, and the organization of the discrete layer of pyramidal cells which is thin enough to facilitate insertion of microelectrodes for observation.

A difference in electrical potential across the excitable membrane of a nerve cell is a universal characteristic. Decrease of this difference, known as depolarization, leads to discharge or "firing" of the cell. An increase in potential difference is called hyperpolarization, and is generally accompanied by decrease or cessation of firing of the cell. It is believed that certain inhibitory mechanisms keep the nervous system in orderly action.

Investigations have revealed two fundamental classes of mechanisms of convulsive action. In one, the action is precipitated by afferent stimulation via the fornix. In these cases, the discharge is repetitive, with depolarization sustained for some time and becoming cumulative to a point where the cell can no longer fire. The second type of mechanism was discovered by severing afferent nerve trunks and testing the hippocampal cells after sufficient time had elapsed to allow the afferent fibers to degenerate. At this stage, stimulation of the fornix activates only afferent fibers. This activation, in turn, excites recurrent collaterals, while hyperpolarizing the pyramidal cells and inhibiting them. A rebound effect occurs after stimulation has ceased, accompanied by a period of convulsive activity.

This finding supports the theory that in clinical epilepsy, convulsions are not necessarily triggered by an excessive discharge of neurons but, in some situations, may be caused by excessive inhibition or lack of activity in certain parts of the neural system. (Drs. Eric Kandel and Alden Spencer, NIMH. Reported at meeting of the American Association for the Advancement of Science.)

LOCALIZATION OF PROCREATIVE FUNCTION IN THE BRAIN

Although the brain has been extensively explored by electrical stimulation, little has been learned until recently about the neural mechanisms involved in such an important function as sexual arousal. Knowledge of the cerebral localization of this function would be of value in a further search to identify structures involved in related manifestations and in social behavior conducive to the preservation of the species.

Earlier studies identified certain positive loci for such activity along a major pathway of the limbic system passing through the septum, the medial preoptic region, and the lateral hypothalamus. Further observations reported this year indicate that positive loci also exist in parts of the anterior and midline nuclei, along the course of the mammillo-thalamic tract, at points impinging on the mammillary bodies, and in the ventral tegmentum.

The implications of these findings are of considerable interest, as the mammillo-thalamic tract, which is not found in the reptilian brain, is confined to the mammal, and attains its greatest size in man. Heretofore the mammillo-thalamic tract has been looked upon as one of the silent areas of the brain, for which no known function has been established. In addition to the mammillo-thalamic pathway, the medial part of the medial dorsal nucleus has also been shown to be specifically involved in sexual arousal. It has been known that an important neural channel exists between the medial dorsal nucleus and the frontal lobes of the brain, and that the frontal lobes play a role in anxiety and tension states.
(Dr. Paul D. Maclean, NIMH. Reported to the Federation of American Societies for Experimental Biology.)

NEW METHOD FOR MEASURING CBF

A new technique, utilizing the radioactive gas krypton, for rapid continuous measurement of the flow of blood through the brain has been devised by a team of scientists at the Aviation Medical Acceleration Laboratory, U. S. Naval Air Development Center, Johnsville, Pennsylvania, in collaboration with scientists from NIMH. The method represents a significant advance over the standard nitrous oxide technique in that it makes possible the measurement of total blood flow minute by minute, with detection of abrupt changes in cerebral circulation as they may occur.

In the studies reported, the gas was inhaled by ten normal young men and measurements of krypton in the brain were taken with a scintillation counter precisely located with respect to the brain

and shielded to exclude radiation from other body areas. Results showed close correlation with those obtained by the nitrous oxide technique.

The new method broadens possibilities for more detailed analysis of changes in the rate of blood flow through the brain, with possible implications this may have for both normal and abnormal mental states.
(Drs. Benjamin Lewis, Richard Wechsler, and W. Budd Wentz, U. S. Naval Air Development Center, Johnsville, Pennsylvania; and Drs. Louis Sokoloff and Seymour S. Kety, NIMH. Reported in the Journal of Clinical Investigation.)

DIRECT CURRENT CHANGES ON CORTEX

Direct current (D.C.) changes on the surface of the cerebral cortex following sensory stimulation have been variously reported. These changes are quite distinct from the fast electrical changes observed by conventional electroencephalographic techniques. Their interest lies in their possible, though as yet undetermined, relation to sensory function.

Using a compact, sensitive and stable non-polarizable calomel electrode of his own design, and special D.C. amplifiers, an Institute investigator has developed a method for recording these phenomena in the lightly anaesthetized and in the conscious animal. He has shown that these D.C. changes are highly sensitive to experimental conditions; but that, with proper care, they can be regularly obtained even in the conscious animal. The characteristics of these changes have been defined. The response to auditory stimulation in various areas of the brain has also been studied. The method represents a definite technical advance in the study of D.C. changes on the surface of the brain and their relation to the processes of attention, sensory discrimination, and the effects of drugs influencing sensory function.
(Dr. R. G. Gumnit, NIMH. Reported to the EEG Society and in the Journal of Neurophysiology.)

PHYSIOLOGIC FUNCTIONS OF SLEEP AND DREAMING

In recent years evidence has accumulated that dreaming is not an occasional and unpredictable occurrence, as had been commonly believed. This assumption gave way before the discovery that dreaming is associated with a specific pattern of brain waves and eye movements which can easily be recorded without disturbance of sleep. The application of this technique disclosed that dreaming is a highly regular and intrinsic part of normal sleep, occurring in scheduled periods over the night, each lasting from 5 to 70 minutes, and taking up an average of 20 percent of sleeping time. That dreaming is

necessary to well-being is suggested by the findings that, if a sleeper is prevented from dreaming over a series of nights by being awakened whenever he begins to dream, there is a progressive increase in the number of his dream attempts, and he makes up for the dreaming lost by dreaming more when allowed to sleep without interruption.

These findings have been confirmed at the Institute, and additional studies have been undertaken to further clarify the physiological accompaniments and functions of dreaming. It has been demonstrated that dreaming is associated with striking changes in other physiological indices, such as respiratory rhythm, heart rate, and skin temperature. The extent of this pervasive physiological upheaval seems to be variable and roughly predictable, depending upon the duration of sleep and the degree of fatigue of the dreamer. It may relate to whether, or how clearly, dreams are remembered on awakening. Continuing studies will attempt to answer such questions as how the dream experience is related to the depth of sleep, and whether derangements in the sleep and dreaming patterns might contribute to the precipitation of mental illness.
(Drs. Frederick Snyder and David Hamburg, NIMH. Reported to the American Psychiatric Association.)

PSYCHOPHARMACOLOGY

The psychoactive drugs discovered in recent years, which affect mental states and behavior, have provided the scientist with a dramatic new tool not only for clinical application in therapy but for the elucidation of brain function. Such drugs include the tranquilizing and energizing drugs which are utilized in the management and control of mental disorders. An understanding of their mode of action, as yet not fully understood, may throw light on the processes that mediate behavior. Another class of drugs, the psychotomimetic agents, produce transient psychotic-like states. Under their influence, normal people experience such typical manifestations of mental illness as hallucinations, delusions, and feelings of unreality and dissociation. Solution to the question of how these psychotomimetic compounds work on the metabolism of the brain and nervous system should provide significant clues to what happens in true psychotic states. An extensive research program in psychoactive drugs is underway at the Institute, and, with its support, at other research centers. The research involves both clinical evaluation of such drugs and studies of their basic mechanisms of action.

PSYCHOPHARMACOLOGY SERVICE CENTER

The Institute's Psychopharmacology Service Center stimulates and coordinates clinical and basic research on psychoactive drugs. It is expanding support for early clinical drug-screening activities, and increasing the number and capacity of clinical facilities available for testing such compounds after adequate animal screening by the pharmaceutical industry. An active program also has been undertaken to provide scientific information to investigators conducting psychopharmacological research.

A cooperative drug study was launched toward the end of the year in nine hospitals. Data will be compiled on the comparative effectiveness of three "tranquilizing" drugs--chlorpromazine, fluphenazine, and thioridazine. Investigators report that fluphenazine, a high potency phenothiazine derivative which is 100 times as potent as chlorpromazine, produces a high incidence of neurological side effects. Thioridazine, equal in potency to chlorpromazine, seems to produce almost no side effects. Fluphenazine was developed by both the Squibb and the Schering Pharmaceutical Companies, thioridazine by the Sandoz Pharmaceutical Company. The cooperative drug study, utilizing a wide variety of clinical settings, is expected to provide results of broader value than could be achieved by a single study in any one hospital. A data-processing unit has been established at George Washington University to analyze material from this and other research projects.

PHENOTHIAZINE DOSAGE STUDY

A new contract between the Psychopharmacology Service Center and Temple University, Philadelphia, is expected to help investigators determine optimum dosage for chlorpromazine, one of the most commonly used phenothiazines. The University's Research Institute will synthesize metabolic products of this potent tranquilizer in order to provide tools for a better understanding of the relationship between its metabolism in the body and its therapeutic action. These metabolic products have been tentatively identified in microscopic quantities in the urine of patients and animals treated with chlorpromazine, but larger quantities of known chemical structure are required for precise identification. The synthetic metabolites will be used as standards of comparison at hospitals where the excretion rate and characteristics of phenothiazine metabolites are under study.

NEW DRUG
ASSAY METHOD

Research into the clinical efficacy of antidepressant drugs will be speeded up by a new chemical assay method developed by scientists at the NIH. An unusual chemical substance, kynuramine, synthesized by a new and rapid route by NIAMD scientists and made available to researchers through a contract negotiated by NIMH's Psychopharmacology Service Center, has been put to use for measuring the activity of the enzyme monoamine oxidase which plays a significant role in body metabolism. This enzyme acts upon pharmacodynamic amines and exercises a regulatory function peripherally as well as within the central nervous system.

Some of the drugs that have proved to be useful in the treatment of depressed patients are also known to have an inhibiting effect on the action of monoamine oxidase. It is not known whether there is a relationship between the inhibiting effect of these drugs on monoamine oxidase and their effectiveness in the treatment of depression. This is an area of research, however, in which there is great interest and vigorous investigation at the present time.

The new technique will greatly facilitate such research efforts. Procedures previously used for measuring monoamine oxidase enzyme activity depended upon difficult and somewhat tedious measurements of either tissue oxygen uptake or substrate amine disappearance. Cumbersome analytical methods are involved in the measurement of amine disappearance. The measurement of oxygen uptake requires manometric assay procedures which also have certain limitations. The new assay using kynuramine utilizes a spectrophotometric method which makes it possible to determine rapidly and accurately the disappearance of kynuramine or the formation of 4-hydroxyquinoline (to which kynuramine is converted as a result of monoamine oxidase activity.) The new method can be applied to crude tissue extracts and appears to be a highly suitable tool for the assay of monoamine oxidase activity.

The new method was developed by NIH scientists. It was made possible by a contract-grant from the National Institute of Mental Health through its Psychopharmacology Service Center. Scientists in NHI and NIAMD obtained the first sample of the rare substance, kynuramine, by a novel and rapid synthetic route from tryptamine. Through the contract which the Psychopharmacology Service Center had negotiated with the Regis Chemical Company, it was possible to promptly prepare a

substantial amount and samples of kynuramine were made available to the NIH workers. The Regis Company was also able to make enough of the compound to offer it for sale to other research investigators.
(Drs. Herbert Weissbach, Thomas E. Smith, and Sidney Udenfriend, National Heart Institute, and Drs. John W. Daly and Bernhard Witkop, National Institute of Arthritis and Metabolic Diseases. Reported in the Journal of Biological Chemistry.)

NEW POWERFUL PSYCHOTOMIMETIC DRUG

Psilocybin is a new synthetic pharmacological compound structured after the active ingredients in an intoxicating mushroom long used by the Indians in Mexico. Its ability to produce hallucinations similar to those experienced in schizophrenia makes it a drug of great research interest. Recent studies have explored the range of its effects, with emphasis on dosages up to 36 mg. Healthy volunteers were used to get a clear picture of drug action unobscured by psychotic symptomatology. In these ranges, the drug was found to produce perceptual, affective, cognitive, behavioral, and autonomic responses qualitatively similar to those previously described with lysergic acid diethylamide, but a greater degree of impairment of cognition.

Twelve of 14 subjects reported visual hallucinations, 9 reported visual delusions, 2 auditory delusions, 10 described body image distortions. Emotional reactions ranged from marked euphoria through anxiety and depression. Marked impairment in verbalizing and disorganized thinking was evidenced. The possibility exists, investigators state, that this may have been related to dosage or personality difficulties.

The studies underscore the potential hazard in use of psychotomimetic drugs as therapeutic agents in outpatient settings. Acute stages of excitement, depression with suicidal ideation, and impairment of judgment may occur with these drugs, even in a carefully supervised setting. The danger of self-administration and inadequate supervision could compound such occurrences. Further research is indicated regarding the site of action of hallucinogens, relationship between personality, dosage level, and total behavioral pattern, and their possible prognostic value in assessing susceptibility to mental illness.
(NIMH grantee Dr. Sidney Malitz, Department of Experimental Psychiatry, New York State Psychiatric Institute. Reported in Comprehensive Psychiatry.)

METHOD FOR CLASSIFYING PSYCHOTOMIMETICS

Further evidence that psilocybin induces a temporary psychosis by acting on the same mechanisms within the brain as does lysergic acid diethylamide (LSD-25) has been obtained by investigators at the Institute's Addiction Research Center. The evidence was provided by studies showing that cross-tolerance developed between the drugs. Such studies previously have been used to establish the biologic similarity of synthetic narcotics to morphine.

Patients who received LSD daily for periods of 6-12 days showed little reaction to LSD, and at the same time showed a markedly diminished reaction to psilocybin. Conversely, patients made tolerant to psilocybin by treatment for the corresponding period of time showed markedly reduced reactions to LSD. The research suggests that studies of tolerance and cross-tolerance may be useful in classifying psychotomimetic drugs into definite groups. Within a group, the drugs should show a high degree of cross-tolerance, with little cross-tolerance between drugs from different groups. If so, this might lead to correlations between psychotomimetic effect and biochemical or neurophysiological processes in the brain, eventually pointing the way to an explanation of how these drugs create psychic aberrations.
(Drs. Harris Isbell, A. B. Wolbach, A. Wikler, and Mr. E. J. Miner, NIMH Addiction Research Center. Reported at regional meeting of American College of Physicians, Lexington, Kentucky.)

EFFECTS OF PSYCHOTOMIMETIC TRYPTAMINE DERIVATIVES

The mode of action of the psychotomimetic drugs which induce transient mental changes in man is of great theoretical and applied interest. The theoretical interest derives from the need to understand the chemical changes a drug undergoes in the body, and the effects of the compound, or its metabolic products, on the brain. The applied interest rests in the hope of antagonizing the effects of a drug through a biochemically related compound. A new type of psychotomimetic compound has been investigated at the Institute by a research worker who first observed the striking transient mental changes induced in man by dimethyl and diethyltryptamine 4 years ago in Hungary. The interest of these compounds lies in their chemical structure, since both are closely related to indole compounds (such as serotonin) known to occur naturally in the animal body and in the brain.

His studies have shown the endogenous conversion of diethyltryptamine into a more active derivative (6-hydroxyldiethyltryptamine). This finding provides one of the very few known instances of the endogenous conversion of a psychotomimetic compound into a <u>more</u> active rather than a <u>less</u> active

derivative in the animal body. Furthermore, joint studies with a behavioral scientist have shown that the rate of this conversion differs from animal to animal in the same species, and that this difference in rate corresponds to differences in behavioral threshold for the parent substance. The higher the rate of biotransformation to the 6-hydroxy derivative, the less of the parent substance was required to produce a given behavioral effect in a particular animal. These findings provide a sound experimental setting for combining behavioral techniques with metabolic exploration in the individual animal.
(Drs. Stephen Szara and Eliot Hearst, NIMH. Reported at meetings of the Federation of American Societies for Experimental Biology and the American Psychological Association.)

EFFECT OF SECOBARBITAL ON ATHLETIC PROCESS

Numerous laboratory investigations have shown that barbiturates and other drugs alter performance on psychomotor and mental tests. Recent studies were designed to determine the effects on judgment of amphetamine and secobarbital. These drugs have long been used as a stimulant and a sedative, respectively. By comparing an athlete's actual performance with his personal judgment of his performance, it was possible to examine a complex process of judgment in a situation in which performance and judgment were matters of personal importance.

Fifteen college swimmers swam 360 time trials receiving 100 mg. of secobarbital, 14 mg. of amphetamine, or a placebo on different occasions. Each was a highly trained athlete in midseason form who was skilled in estimating performance time. After each trial the swimmers estimated their performance time and gave a detailed evaluation of their performance. Secobarbital significantly impaired performance, and amphetamine significantly improved it. The subjective data showed that secobarbital produced distortion in judgment. After taking secobarbital, swimmers thought their performances were unusually good, whereas in fact they were unusually bad. The effect of amphetamine on judgment was not conclusive. The profound impairment in judgment due to secobarbital was produced when the subjects swam in groups of three. Solo swimmers taking amphetamine thought their performance was about the same after amphetamine as after placebo. Actually, amphetamine had significantly improved it.
(NIMH grantees Drs. Gene M. Smith and Henry K. Beecher, Harvard Medical School. Reported in the Journal of the American Medical Association.)

PSYCHOMOTOR EFFECTS OF MEPROBAMATE

Although administration of meprobamate (a mild tranquilizer developed by Wallace Laboratories) has not been found to affect driving ability for normal subjects adversely, a slight but significant decrease in psychomotor activity performance has been found among anxious neurotics. Research has disclosed that with subjects psychiatrically diagnosed as anxious neurotics, chronic dosages of meprobamate led to slowed reaction time in simulated driving at high speed, accompanied by a slight lowering in accuracy. Investigators suggest that increased ability to concentrate, produced by the meprobamate, may have resulted in a concomitant decrease in ability to shift attention. Since these findings differ from those observed with normal subjects, they suggest the possibility that this effect of meprobamate may be specific to anxious neurotic patients.
(NIMH grantees Drs. Leonard Uhr, John Pollard, and James Miller, University of Michigan. Reported in Psychopharmacologia.)

DRUGS OF LIMITED VALUE WITH DISTURBED CHILDREN

Reports from recent studies have raised questions regarding the effectiveness of the use of tranquilizing drugs, successful with adults, in treating children with behavioral problems. Treatment with meprobamate and prochlorperazine have been observed to provide no evidence for the superiority of either drug over placebo. Prochlorperazine was developed by Rhone-Poulenc Laboratories in France, and marketed in the United States by Smith, Kline, and French. More recently comparative studies by the same investigators have been conducted to determine the effectiveness of psychotherapy alone and in conjunction with a new agent, perphenazine, or placebo in treating neurotic or hyperkinetic (overactive and distractible) children. Perphenazine was developed by Schering Laboratories.

Whether on perphenazine or placebo, two-thirds of the neurotic group, and one-third of the hyperkinetic group demonstrated significant improvement. The findings suggest that the diagnostic differentiation between neurotic and hyperkinetic is important in predicting outcome of patients receiving brief outpatient treatment.

Investigators noted that the prompt and usually satisfactory response of neurotic children to brief psychotherapy left little room for any but the most remarkable drug to demonstrate its effects. More effective pharmacologic agents are being sought for use with hyperkinetic children who do not respond so well to psychotherapy.
(NIMH grantee Dr. Leon Eisenberg, Johns Hopkins University. Reported at annual meeting of the American Psychiatric Association.)

CLINICAL STUDIES IN A MENTAL HOSPITAL SETTING

Drug studies carried out in the Institute's Clinical Neuropharmacology Research Center at Saint Elizabeths Hospital have centered on problems related to the management and treatment of the chronic schizophrenic patient, a dominant problem in a mental hospital.

A comparative drug study, involving two tranquilizers and a placebo, was initiated simultaneously on six wards of the William A. White Service. The investigation was also planned to examine in detail, and with an emphasis on quantitative assessment, the effects of nondrug factors known to affect profoundly the efficacy of drugs in a typical mental hospital setting. Essentially, the study thus aimed at: (1) determining the effect of physical environment on responsiveness to drugs; (2) determining the effect of various types of nursing care (and particularly nursing in small groups) on drug response; (3) the cultivation of native therapeutic and research skills often found, but ignored, in ward personnel; (4) the codification of a number of questions commonly entering into a drug trial, including selection of patients, selection of drugs, selection of experimental design, technique of drug administration in a blind study, assessment of side effects, management of adequate laboratory control, and the comparative examination of a number of clinical evaluative techniques; (5) the monitoring of attitudes toward research; and (6) the institution of data reduction and statistical techniques best suited to such a drug study. The dimensions of the study were kept within the limits of applicability and feasibility to a State hospital setting, so as to make possible a retesting of the conclusions throughout other services of Saint Elizabeths Hospital and other State hospitals.

A quantitative analysis of data is now proceeding. Several trends, however, are clearly apparent. A coordinated program of clinical investigations proceeding simultaneously in a number of wards of a mental hospital building (rather than a single research service) can profoundly, and with great economy, contribute to the raising of both therapeutic and research skills of personnel throughout such a building. Nursing in small groups, structured around the skills of attendants and of cooperative patients, can act as a pacemaker in changing the therapeutic climate of a chronic ward, and usefully contribute to the rehabilitation of patients. Such a regimen also reduces consumption of tranquilizers on a ward. The use of suitably designed documents enhances the observer skills of personnel, while at the same time providing quantitative data which can be subjected to machine analysis.

The wider application of the study was shown by its replication on a more extended scale in 126 very deteriorated patients. Group nursing techniques and drug research designs developed in the Service have also been applied in other parts of Saint Elizabeths Hospital. With the cooperation of the Medical Arts Section, National Institutes of Health, an extensive teaching and demonstration exhibit was prepared, based on the initial study, and was exhibited at meetings of the American Psychiatric Association and the American Medical Association.
(Drs. A. Hordern and M. Hamilton, Visiting Scientists, NIMH; Dr. J. Lofft, NIMH; and Dr. F. N. Waldrop, Saint Elizabeths Hospital.)

CHILDHOOD STUDIES

Because of the significance of early formative years for later adjustment, much of the behavioral research of the Institute is focused on child development and patterns of family living and social interaction that play a determining role in influencing the direction of growth.

SEEKING CLUES TO CHILD BEHAVIOR

A unique series of studies to determine the predictability of certain child behavior patterns, based on a knowledge of how he reacts at birth and of factors in the parents' marriage relationship, has been underway this year at the Institute's Biosocial Growth Center. The studies are part of a long-term research program which will also concentrate on the child's extrafamilial relationships, and an evaluation of the influence of other events occurring during infancy and early childhood.

The prenatal family environment is being charted with the cooperation of 40 newlywed couples, who have been observed both at home and at the Institute. Techniques that have been developed to provide information include experimental situations, psychodrama, and questionnaires and interviews. As children are born into these families their behavioral patterns will be observed from the earliest manifestations of infant personality, with special regard to feeding habits and skin sensitivity. Earlier studies have indicated that these characteristics may provide significant clues for the prediction of subsequent behavior.

INTERRELATION OF MATERNAL AND CHILD BEHAVIOR

The influence of a mother's behavior on child development has been examined over a period of years and has recently been reported in a study on environmental influence on personality. Findings are based on data collected in California on 54 subjects from birth to adulthood, and analyzed at the Institute.

Studies on the consistency of attitudes over a period of time show that a mother's acceptance or rejection of a child remains relatively the same from infancy through preadolescence. Attitudes of permissiveness or control may vary, however. Child behavior as to extraversion or introversion is rather consistent through childhood; it is not so as regards adjustment or maladjustment.

Findings suggest that parental attitudes of permissiveness or constraint may be less important factors in relation to a child's over-all adjustment than has been thought. Much more important appears to be the degree to which a child is accepted or rejected by his mother.

Family socioeconomic status also appears to be less related to the child's behavior than his mother's attitude toward him, though it may play a part, along with financial stress and health, in determining maternal behavior. How a mother reacts to her son during infancy is more closely related to his adjustment at 9 and 12 years of age than is his own infant behavior, suggesting that early maternal patterns may be more predictive of the child's future adjustment than is his own early behavior.

In the families studied, boys appeared to have been more influenced by the way they were handled by their mothers in infancy than were girls. Girls seemed to be more affected by current maternal behavior, suggesting that girls respond more to their current interpersonal situation, while boys tend more to reflect past relationships.
(Drs. Nancy Bayley and Earl S. Schaefer, NIMH. Reported at the 1960 Regional Research Conference on Child Development of the American Psychiatric Association, at the University of Iowa.)

ALLOCATION OF PARENTAL RESPONSIBILITIES

Most theories of personality development have been based on a model of a family in which the role of mother and father are necessarily differentiated, with mother specializing in providing emotional support and father in imposing restraints. Present research, however, on social class and parental responsibility indicates that both middle and working-class variations on this theme are sufficiently great to compel a more precise formation.

Previous data have disclosed decidely different emphases in middle- and working-class values for children. According to these findings, the dominant motif of middle-class parents' values is that the child develop his own standards of conduct; of working-class parents, that the child's actions be reputable. Further studies by the same investigators reveal that, in a sizeable proportion of middle-class families, it is mother who takes primary responsibility for imposing constraints on sons, and father is at least as supportive as mothers. Fathers are seldom as supportive of daughters as are their wives.

While it would be an exaggeration to state that middle-class fathers have abandoned the prerogatives of authority in favor of being friend and confidant to their sons, this research suggests that the historical drift is probably toward primary emphasis on support. It supports the conclusion that today in middle-class families, mothers' and fathers' roles are not sharply differentiated. To the degree that they are, a new division of responsibilities is developing, with each parent being most supportive of children of his or her own sex.

Mothers' and fathers' roles are more sharply differentiated in working-class families, with the children almost always feeling that mother is the more supportive parent. Yet fathers do not specialize in setting limits, even for sons. In most working-class families, mother raises the children, father provides the wherewithal. The studies reported indicate that this pattern of role-allocation is probably far more prevalent in American society than has been recognized by the formal theories of personality development.
(Drs. Melvin L. Kohn and Eleanor E. Carroll, NIMH. Reported in Sociometry.)

BIRTH ORDER AND SCHIZOPHRENIA

Data supporting a relationship between birth order and schizophrenia, which has been hypothesized by other investigators, have been produced in a study of hospitalized schizophrenics conducted by an NIMH investigator. Among a cross-section of female schizophrenic patients, it was found that significantly more patients came from the last half of their sibling group than from the first half.

If a relationship between birth order and incidence of schizophrenia does in fact exist, as the studies indicate, the problem of finding the cause yet remains. One possibility which the investigators discuss is that the apparent effect of birth order may be merely an artifact of the relationship between the increasing age of the mother and incidence of schizophrenia. Both physiological and psychological explanations are possible. Explained in

terms, it is possible that increasing age and large numbers of children may affect the intra-uterine environment in such a way as to leave the child with a physiological predisposition to the disease. Explained in psychological terms, it is possible that younger children coping with anxiety may have less access to their parents.
(Dr. Carmi Schooler, NIMH. Archives of General Psychiatry, in press.)

CHILDHOOD ENURESIS

Evidence disputing the traditional view that childhood enuresis is a symptom of emotional instability has been produced by Washington University and the St. Louis County Health Department. In a study of 830 children at the 8- and 9-year age level regarded as bed-wetters, assessment made on the basis of information provided by teachers, mental health personnel and families show very low correlation between this habit and other symptoms of psychological disturbance. In no instance did the study show to any significant degree that enuretics have greater maladjustment than nonenuretics, or that enuresis can be regarded as more closely associated with emotional symptoms than is thumb-sucking. The conclusions do not hold for adult enuretics, in whom a correlation with psychopathology has been found. They serve only to show that there is little justification for assuming that bed-wetting continued into the ninth year suggests emotional disturbance.
(NIMH grantee Dr. F. Tapia, St. Louis County Health Department, J. Jekel, Washington University School of Medicine, and Herbert R. Domke, Allegheny County Health Department, Pittsburgh. Reported in The Journal of Nervous and Mental Disease.)

AGING

With the increasing complexity of society, certain specific problem areas such as aging have emerged that have relevance to mental health and mental illness. With many other groups, the Institute shares a continuing concern for these areas and seeks to initiate and coordinate research programs that will not only provide fuller understanding of the processes involved, but will to some extent help to restore to society some of the human potential now lost.

NEW PSYCHOLOGICAL THEORY ON AGING

Much recent research has focused on the psychosocial aspects of aging, in an effort to gain further insight into the nature and extent of psychological changes that occur in the aging individual. A major study, reported on this year, has been conducted in Kansas City by

the Committee on Human Development of the University of Chicago, on a grant from the Institute. Observations have been made over a 5-year period on 211 persons, including a panel of healthy adults between the ages of 50 and 70, and a group of ambulatory old people between the ages of 70 and 90.

From the intensive study of findings there has emerged the theory of "disengagement," in which aging is seen as an inevitable mutual withdrawal or disengagement, resulting in decreased interaction between the aging person and others in the social system to which he belongs. The process may be initiated by the individual or by others around him, and he may withdraw more markedly from some classes of people and remain relatively close to others. His withdrawal may be accompanied from the outset by an increased preoccupation with himself. Certain institutions in society may make this withdrawal easy. But when the aging process is complete, the equilibrium that existed in middle life between the individual and his society has given way to a new equilibrium characterized by a greater distance and an altered type of relationship. The investigators theorize that some intrinsic change may occur with age which is the analog of maturation in children, which brings about this loss of involvement or disengagement from the environment. The disengaged state appears to be an almost universal end point, and it appears to be irreversible.

The theory differs from the traditional theory of aging which assumes that the aged person is isolated through desertion by others in his social structure, and that the constricted world of the aged is not of his own choosing. The disengagement theory suggests that he is not merely subjected to a shrinking universe, but participates in creating it. The hypothesis of disengagement indicates a reappraisal of some of the common assumptions associated with the "activity" theory of aging. For example, a widely held concept that successful aging consists in continuing middle-aged activities as much as possible may not be valid. Many old persons, the study showed, do not desire an extension of continued usefulness, or the development of new interests and new contacts. They find equal satisfactions in their more confined spheres of activity.

In the process of disengagement, specific changes were observed to take place, contributing to the aged person's becoming less bound to the social system of which he is a member. A shift in perception occurs in the sixth decade which may reflect both a withdrawal of intense emotional attachment to objects and people, and a beginning of anticipation of the aged state. This shift is accompanied by a constriction in the variety of social interactions undertaken, and is followed shortly after by a reduction in the number of hours spent in the company of others.

Concomitantly, relationships gradually become redefined so as to be less like those of family, work, and voluntary associations and more like those of recreation. A change in behavioral motivation becomes apparent. Desire for approval and love, the typical rewards of work and kinship, strikingly lessen with old age, and a new freedom of choice of rewards emerges, with a tendency to select short-run gratification such as are found, for example, in recreational activities. The theory, postulating a lessened concern for social approbation and desire for esteem in advancing years, helps explain the self-centered and idiosyncratic styles of behavior often noted among the ambulatory aged.
(NIMH grantee William E. Henry, University of Chicago. Reported in Sociometry by Dr. Elaine Cumming, Project Coordinator.)

RESEARCH SEMINAR ON AGING

In the summer of 1960, an International Research Seminar on the Social and Psychological Aspects of Aging was held in Berkeley, California, in conjunction with the Fifth International Gerontological Congress. Institute support was provided for travel grants for foreign participants and for publication of the proceedings, and the program was jointly developed by the Professional Services Branch of the Institute and the Division of Gerontology of the University of Michigan.

Research presented and discussed at the Seminar included recent studies on the psychological capacities of the aged, including perceptual and thought processes; personality, life styles, and social roles, focusing on how older people participate in social processes and how this changes with age; mental health and rehabilitation; and health aspects of income, employment and retirement.

ADAPTIVE CAPACITIES OF AGED

Changed capacities and changed circumstances that characterize old age impose limitations and require reorganizations and substitutions in behavior. The predictable structure of everyday living in terms of the routine responsibilities and motivations relating to family, work, and community breaks down, and a new structure must be built to replace it. A physical organism, itself undergoing change, must deal with often major changes in its social environment.

This study focused on the adaptive capacities of a group of healthy aged men to their changed social environment. The relative good health possessed by all in the sample population was not correlated with a homogeneity of well-being in personal

social functioning. Both hopeless resignation and creative involvement were found among persons studied. Some had withdrawn to routines and self-centeredness, others were engaged in varied and absorbing activities.

Previous educational and occupational levels and ethnic classifications did not appear to affect the capacity of an aged person for social functioning. Though at the height of their careers the ways of life of a professional person and an unskilled worker had differed markedly, by the age of 70 the elements of day-to-day activities and attitudes about being old had merged into similar general patterns. This "leveling" results partly from the tendency for these men to drop their specializations, find similar hobbies, use common recreations afforded by culture, and engage in many "maintenance" activities of the household. Inner resourcefulness was by no means solely the property of the more educated.

In only one respect did educational factors appear to relate clearly to present adaptation: there was a significant association between education and relationships with children. Conflict and feelings of rejection were found more frequently between the less-educated men and their children. Studies of individual cases suggest that discrepancies in educational and cultural level of parent and grown children, rather than low education per se, characterize the families for whom conflict is accentuated. Extensive cultural displacement from conditions of early childhood also tends to handicap certain adjustments.

Success in working careers interpreted as evidence of adaptability and striving appeared to be predictive of greater adaptability to the stresses of aging. Men who advanced in their jobs showed different adaptations from those who did not move upward--having more complex and varied living patterns and enjoying better relationships with their children in old age.

In studying the processes by which the subjects had retired from active work, it was evident that fewest difficulties resulted when the decision to retire had been made voluntarily by the man himself, or was controlled institutionally. Those who arrived at retirement through conflicting pressures suffered most ill effects.

Immediate environmental factors were, as expected, found to be closely related to the aged persons' behavior and attitudes. Loss of loved ones, loss of income and cultural displacement considerably affected adjustment. The degree of loss in the total structure of psychological supports, rather than loss in any one area, was critical for functioning.
(Dr. Marian Yarrow, NIMH. Reported at the International Research Seminar on Social and Psychological Aspects of Aging, Berkeley, California.)

LOSS OF APTITUDES IN AGED

An evaluation of the reasoning processes of the aging individual, made possible through problem-solving experiments designed to identify the processes accounting for failure or delayed success, has provided specific descriptions of some of the mental deficits associated with aging.

In test situations, the aged person was unable to achieve a clear identification of the goal of the problem before embarking on random trial-and-error exploration. College freshmen and sophomores, on the other hand, did not manifest this deficiency to a serious degree on any occasion, whereas all old subjects exhibited it on some problems and most old subjects were frustrated or impeded by it on all.

A lack of organization in search for clues or helpful information was also evidenced, as well as excessive redundancy and repetitious eliciting of the same items of information. Other peculiarities of the aged person's performance included failure to develop strategies, apply those suggested to him, and draw appropriate inferences. The results of the study emphasize the urgency of investigating the feasibility of retraining in the basic heuristic processes which appear to deteriorate so sharply in the process of aging.
(Dr. Edward Jerome, NIMH. Reported at the International Research Seminar on Social and Psychological Aspects of Aging, Berkeley, California.)

STYLES OF LIFE AND SUCCESSFUL AGING

An intensive study of data on some of the 20 aged persons in Kansas City has been conducted in an effort to delineate various styles of life found among older people and to evaluate the degree of successfulness with which these individuals cope with aging. In the process of studying these cases, a means of defining how successfully an individual ages has been developed which involves a new approach to analysis of the data procured.

The approach is based on observation of the social system in which a given individual functions--the dimensions and structure of his social life space, his modes of interaction within it, his attitude toward it, the satisfaction it provides, and its degree of stability over a period of time. Evaluations are made of two aspects of the social systems of the individuals studied. One concerns the amount of "output" or work a person contributes to the system, and how much "input" or work of others it takes to keep him going. If there is a balance on account, or a plus value on the output side, the individual is said to be autonomous; but if it takes more energy to maintain him than he puts out for the system, he is said to be dependent. The other aspect of the individual social system to be judged in defining the success or lack of success of an individual in coping with aging, is its probable future course. Will it persist, change, or collapse--in short, is it regarded as persistent or precarious?

Utilizing these four characteristics, and the relative degree to which they apply to an individual, a judgment can then be made regarding the extent to which aging can be regarded as successful. Four combinations are possible: autonomous-persistent (successful aging); autonomous-precarious (potentially unsuccessful aging); dependent-persistent (semi-successful aging); and dependent-precarious (unsuccessful aging).

Through this method, cases can be ranked within each of the four categories. The extreme cases of unsuccessful aging would lie outside the four categories, and would be exemplified by institutionalization or suicide.

This approach to measuring the success with which people age does not involve such factors as the happiness of the subjects or the amount of satisfaction they draw from life.

Determinations of the autonomy-dependency of a social system of an individual, while complex, are made solely on the basis of the subject's social system and without regard to his health or economic status.

This method of determining the extent to which an individual may be aging successfully is independent of any specific theory of aging, but it provides a useful frame of reference in exploring varied aspects of theories of aging.
(Dr. Richard H. Williams, NIMH Professional Services Branch. Reported at the International Research Seminar on Social and Psychological Aspects of Aging, Berkeley, California.)

LIFE SATISFACTION AND SUCCESSFUL AGING

Two indices of life satisfaction which can be used to measure success in aging in terms of the degree of satisfaction a person feels with his present and past life have been developed by the team involved in the Kansas City study. Preliminary use of these indices, which show promise of wide applicability, suggest that life satisfaction is positively related to activity for some types of people but is related to disengagement for significant numbers of others.
(NIMH grantee Dr. Robert J. Havighurst, University of Chicago. Reported at the International Research Seminar on Social and Psychological Aspects of Aging, Berkeley, California.)

AGE AND INTELLECTUAL FUNCTIONS

Investigators at Fordham University have been studying the relationships between performance on a test which measures the brain's ability to perceive and discriminate a rapidly flickering light and brain pathology, age, and intellectual functions. The point at which the intermittent light fuses and is perceived as a steady light is designated as the critical flicker frequency (CFF) for any given individual. Earlier investigators noted that the average CFF of groups with brain pathology is lower than the average CFF of normal groups, and thus CFF has been viewed as a potentially useful indicator of the integrity of the central nervous system. In their work with 50 women residents (ages 74-80) of homes for the aged in New York City, the researchers at Fordham University explored the hypothesis that if there is a correlation between CFF and intellectual functions, the relationship should be evident in old age, and that there should be more pathologically lowered CFF's in old age than at any other time of life. They administered 5 tests of intellectual function to a selected group of 50 elderly women who were free of eye pathology, and also tested their CFF thresholds. Their findings corroborated the suggestions of earlier investigators who worked with a small sample of elderly men. The results of all but one of the tests of intellectual function correlated significantly with critical flicker frequency. Tentatively the investigators ascribe the relationship of CFF and intellectual functioning to reduced central neural efficiency, or "alertness, in old age, which adversely affects both CFF and intellectual functions, more especially comprehension of new ideas and adoption of new work methods.
(NIMH grantees Drs. Armand W. Loranger and Henryk Musiak, Fordham University. Reported in the Journal of Clinical Psychology.)

SOCIAL ISOLATION IN THE AGED

A scientific exploration of the adverse effects of social isolation among 100 residents of a home for the aged revealed that a lack of prior personal interaction hindered the older individual's adjustment to a new environment. Social isolation was defined as "the absence of role relationships which are generally activated and sustained through personal interaction." The amount of contact with relatives, presence of children, length of unemployment period, and mode of living--i.e., alone or with others--indicated the degree of withdrawal. In addition to having difficulty in adjusting, the "isolates" appear to have a disruptive effect upon their new surroundings--especially if the social system involved is highly integrated. The most important factor, the investigators feel, is a loss of social skills and sensitivity to social expectations, leading to disruptive nonconformity. More "isolates" than controls in the study had to be transferred to mental hospitals. (NIMH grantee Dr. Joseph Zubin, Columbia University. Reported by Nechama Tec and Ruth Granick in Social Problems.)

DELINQUENCY

RESEARCH ON PREVENTION

The growing rate of juvenile delinquency, which doubled during the years between 1948 and 1958, has focused alarmed attention on this problem area. With the child population estimated to increase 50 percent in the next two decades, understanding of the factors contributing to juvenile crime and the development of measures to deal with them have become matters of paramount importance to society. The problems are of particular relevance to the field of mental health. Through this strategic discipline, knowledge of many areas may be brought together to be related, tested, and applied.

The NIMH therefore includes this problem among its areas of concern, and during the past year has engaged in and supported numerous research programs designed to provide better understanding of the processes that produce delinquent behavior. While this long-term basic research moves forward, steps have also been taken to increase the effectiveness of community agencies in handling mental and behavioral problems.

COMMUNITY PROJECTS

The elements of an effective prevention and early treatment program on a community-wide or high delinquency-wide area have long been recognized. To make immediate inroads on this problem, the Institute has therefore supported several such trial

programs designed on a sufficiently extensive basis to produce conclusive results. Emphasis has been placed on development of instruments for early identification, careful recording, classification for treatment purposes, and finally, for evaluation.

New York's Lower East Side, whose underprivileged homes produce a disturbingly high number of juvenile delinquents, is the target of the pilot phase of a demonstration project started this year. The project seeks to explore new patterns of services in a circumscribed area which will affect delinquency. How can the families be reached? To what influences will youth respond? New methods of intervention and modes of management, utilizing indigenous value systems and aspirations, are being utilized. How can potential delinquents be identified at a sufficiently early age so that skilled clinical and social services may be brought to bear, in time? With the cooperation of school personnel in the pilot area, measures which are capable of detecting a percentage of children with a high potential for psychosocial disturbance in early childhood will be utilized. Agencies of the Lower East Side Neighborhood Association in cooperation with Columbia University are collaborating in the project, known as Mobilization for Youth.

A second community project supported by the Institute is underway in Boston. Here methods for classifying a sample of all delinquents coming to court, so as to suggest appropriate disposition of treatment, are being developed by an interdisciplinary team of child psychiatrists, psychologists, social workers, and a social scientist. The project is designed to develop instruments which can be applied by partially trained probation officers or other semi-professional workers.

NEW EVALUATIVE TECHNIQUES

Effective evaluation methods are important in early detection of delinquent tendencies. A new technique is now being developed in New York City through Institute support. The method, designed by Dr. Isador Chein, utilizes multiple correlation, with delinquency rates, of a number of factors given by census data for census tracts--such as median incomes, home ownership, racial make-up, morbidity rates. Experience has shown that those scoring high on these indices tend to have high delinquency rates. A multiple service saturation-type delinquency prevention and treatment program is proposed for those tracts in which high correlation exists. As controls, census tracts in the city with very similar characteristics will be used. It is assumed that, if the saturation program is successful over a period of years, the correlation between delinquency rates in this area and the background factors should be substantially reduced,

since unfavorable environmental conditions would be offset by the project. The correlation, presumably, would remain high in the control areas. This approach has promising implications for sociological studies, for, if successful, it could be applied to any effort to effect change in a community.

SUICIDE

Suicide is a major mental health problem in the United States, as in other civilized nations of the world, with approximately 18,000 people in this country committing suicide each year.

The Institute this year has continued its support of the Suicide Prevention Center in Los Angeles, California. This 5-year project utilizes community agencies to obtain referrals of suicidal persons, and public and private facilities in handling the patients. Arrangements have been made whereby the treatment resource reports back to the Center on care provided and on the patient's response.

Comprehensive research on suicidal motivations is being conducted at the Center, which is unique. The purpose of the project is to develop hypotheses about the precipitating causes of, and treatment for, persons suicidally inclined; to obtain psychological, psychiatric, and environmental data by means of interviewing and testing such persons; to diagnose and refer them for treatment, and correlate treatment data with subsequent records; and to evaluate the effectiveness of methods of treatment used in the community.

In a recently published book, The Cry for Help, the Center has described the results of the clinical and research activities of its interdisciplinary staff of therapists and researchers. Work done by the Center will help stimulate other communities to establish suicide prevention facilities and encourage additional basic investigations of the cause of, and effective preventive measures against this serious emotional illness.

RETARDATION

IMPROVING ABILITY TO COMMUNICATE

Procedures designed to improve the ability of severely retarded children to communicate have been developed during the past 3 years by the University of Kansas and the Parsons State Hospital. A variety of ways to stimulate language are utilized in this Institute-supported project which seeks to increase the sociability of institutionalized retarded children, who have been isolated from each other and from the world because they are not able to use language effectively.

Trained aides are instructed on communicating with the children, and cautioned not to expect too little of them. Children are encouraged to talk by being asked questions requiring more than a simple "yes" or "no" answer. It was found that children responded more to one another than to adults. The value of incorporating a system of rewards to induce the child to conform to the social conversational norm is underscored.

PILOT SCREENING PROGRAMS

Phenylketonuria is an inherited disease traceable to an inborn error of metabolism which results in mental retardation. The disorder is caused by the lack of an enzyme needed to metabolize phenylalanine, an amino acid, into another amino acid called tyrosine. As a result, abnormal amounts of phenylalanine accumulate in the blood and urine. Although there is no cure for the metabolic defects, it is now known that retardation can be prevented if a special low phenylalanine diet is instituted early in infancy. Findings have shown that treatment must be started in many cases as early as at age 4 to 6 weeks if it is to be fully effective. Early diagnosis is therefore crucial.

A pilot case-finding project, made possible by an Institute grant, is now underway in the Cincinnati area to screen infants for evidence of this and other metabolic diseases. Based on a diagnostic evaluation survey conducted as part of this project, it is estimated that the incidence of phenylketonuria is one in every 10,000 newborn children. The program utilizes a diaper test adaptation of the standard urine test. Small squares of filter paper are distributed by hospitals to mothers of newly born infants, with instructions that they be placed in diapers when the babies are 4 to 6 weeks old. When dried, the squares are returned to the hospital, where they are analyzed for detection of phenylketonuria, galactosuria (a disorder resulting from an inherited inability to metabolize galactose and lactose normally), and other patterns of aminoaciduria that may result in mental retardation. (NIMH grantee Dr. Helen K. Berry and associates, Children's Hospital Research Foundation and Department of Pediatrics, University of Cincinnati College of Medicine. Reported in Cincinnati Journal of Medicine, Clinical Chemistry and Metabolism.)

A similar early detection program in Los Angeles is attempting to establish the efficacy of the filter paper test in early infancy so that the method can be used routinely. These investigators report that in treating the phenylketonuric patient by diet, the physician is faced with the problem of controlling the diet as carefully as he would for the diabetic patient. They

also advise frequent testing of urine and blood for the presence of high amounts of phenylalanine since the actual amounts of phenylalanine required to maintain normal blood levels in the phenylketonuric patient appear to vary with each patient. (NIMH grantees Drs. W. R. Centerwall and P. B. Acosta, College of Medical Evangelists, Los Angeles. Reported in the Journal of the American Dietetic Association.)

RETARDATION AND FETAL DEVELOPMENT

Disorders which are associated with mental retardation and which occur during fetal development are being investigated in research conducted by a team at the University of Michigan. These studies have considerable interest with regard to the prevention and treatment of mental retardation associated with congenital hypothyroidism or cretinism (a disease resulting from a lack of thyroid hormone during fetal development which affects the cerebral cortex, with accompanying physical and mental disorders). Until recently cretinism was not considered to be an important cause of mental retardation. However, studies of inhabitants in an endemic goiter area in northeastern India seem to indicate that it may be much more important than had been suspected. Much of the work of this group has been devoted to examining hypotheses dealing with this disease. For example, they have demonstrated that although antibodies are known to cross the placenta from mother to fetus, autoantibodies present in the mother are apparently not a cause of congenital hypothyroidism in the fetus. Thyroid hormone can be administered to the pregnant mother in extremely large doses without apparent harm to the mother and yet be successfully transmitted across the placenta to be absorbed by the fetus.

In a specific case, a woman who had previously given birth to two cretins was given dessicated thyroid when she became pregnant for the third time to assist the fetus to develop normally. At the birth of this child, tests showed that the child was normal and that normal uterine conditions had been produced by the administration of thyroid to the mother during her term of pregnancy. Further studies showed that a fetus may have temporary hypothyroidism, while in utero, which results in brain damage. After the child is born, his thyroid gland develops normally. Thus, the child will be normal physically but mentally retarded to the degree of the temporary nonfunctioning thyroid during fetal development.

From this research has come not only an increasing recognition of the need for diagnosing and treating congenital hypothyroidism early, but the actual beginnings of diagnostic methods for

recognizing it at birth or a few weeks afterward. The presence of an umbilical hernia, prolonged cyanosis, or a high birth weight (over nine pounds) have been found to be indicative of congenital hypothyroidism at birth. Within a few weeks other symptoms are observed, such as feeding difficulty, retarded growth, prolonged icterus neonatorum, and respiratory distress with cyanosis.
(NIMH grantee Dr. W. H. Beierwaltes and associates, University of Michigan. Reported in the Journal of the Michigan State Medical Society and the Journal of Clinical Endocrinology and Metabolism.)

DRUG ADDICTION

ADDICTION AND MENTAL DISTURBANCE

Significant evidence linking mental disturbance and drug addiction has been found by investigators at the Institute's Addiction Research Center. A recent study of 270 hospitalized drug addicts showed that 95 percent of the patients had personality characteristics associated with sociopathy ("constitutional psychopathy"). In addition to such sociopathic traits, most addicts also had psychoneurotic, hypomanic (depressed), or schizoid traits. Overt psychoses were rare.

Assessment of these characteristics was based on the Minnesota Multiphasic Personality Inventory (MMPI), a standardized objective test which measures several aspects of personality. All groups showed high elevation on the psychopathic deviate scale, and less than 5 percent of the addicts showed normal MMPI profiles. On the basis of information provided, patients could further be subdivided into three groups, including neurotic, hypomanic, and schizoid. The similarity in the personality profiles of adolescent addicts and adult addicts, as well as the resemblance of the profiles of adolescent addicts to those of nonaddict delinquents, suggests that psychopathology has considerable significance in the etiology of addiction.
(Drs. Harris E. Hill, C. A. Haertzen, and R. Glaser, NIMH Addiction Research Center. Reported in the Journal of General Psychology.)

SUBJECTIVE DATA ON ADDICTIVE DRUGS

Evaluation of the addictiveness of new narcotics has generally been based on objective measurements, such as the ability of the new drug to suppress withdrawal symptoms of morphine or to create physical dependence

similar to that caused by morphine. Little effort has been made to measure subjective responses, in spite of their recognized importance in causing addiction, since reports from addicts were regarded as unreliable and evaluation difficult. Recent studies, however, at the Institute's Addiction Research Center show that subjective responses induced by opiates can be quantitatively measured. Patients receiving drugs, without knowing their identity, were asked a series of simple questions: Did the drug produce any effect? Was it similar to that of any other drug they could name? Were there symptoms such as relaxation and itching caused by morphine? The subjects also rated the strength of the drug. Answers were found to correlate highly with objective measures of drug effects such as constriction of the pupils and intensity of withdrawal symptoms. (Drs. H. F. Fraser, G. D. Van Horn, W. R. Martin, and H. Isbell, NIMH Addiction Research Center. Reported to the Committee on Drug Addiction and Narcotics of the National Research Council.)

REHABILITATING DRUG ADDICTS

Progress in the extremely difficult and seemingly hopeless task of socially rehabilitating drug addicts has been made at the Institute's unique Drug Addiction Demonstration Center in New York City. Work with addicts, their families, and various social agencies in the community has helped a number of addicts abstain from use of drugs for increasing periods of time. Referrals have been made to several employment services and families have been prepared to assist the addict in readjusting to normal life.

The Demonstration Center, established in 1957, is staffed by a director, six social workers, a case-work supervisor, and a psychiatric consultant. It has functioned increasingly as an informational and consultative source, answering inquiries from professional and lay persons. It has extended its influence through consultation with community agencies, participation in public conferences and seminars, and membership on committees interested in formulating programs for the management of addiction.

ALCOHOLISM

The use of alcohol is one of many mechanisms that can be employed to cope with everyday problems of living. Its widespread use in our culture and the stress imposed by its misuse have focused increasing attention on the causes of alcoholism and methods of prevention. The search for answers to such questions as why some people are able to drink alcohol socially while others employ it as a means of escape from problem-solving, leads directly into the psychobiological disciplines of mental health.

Last year, a grant was made to the North American Association on Alcoholism Programs (an organization of alcoholism program administrators in the United States and Canada) for the purpose of establishing an independent Cooperative Commission on the Study of Alcoholism. With the recent appointment of Dr. Nevitt Sanford, professor of psychology at the University of California, as chairman of the Commission, a major impetus has been given the program which will reexamine the problem of alcoholism and develop a blueprint for action by communities.

Meanwhile, scientists at the Institute are investigating the psychosocial dynamics of alcoholic disease. Others are approaching the problem of alcoholic addiction through metabolic and biochemical studies, seeking evidence of biological factors that may have a bearing on alcoholism. Discovery of such factors, if they exist, might eventually lead to medication to counteract them.

ALCOHOL AND NEUROHORMONE CONCENTRATION IN BRAIN

Although the effects of alcohol on the biological system have been widely observed and measured, little has been known about the precise mechanism through which alcohol acts to bring these effects about. An Institute-supported study, designed to test a number of biological factors as possible somatic traits for chronic alcoholism, has now provided an explanation of how the sedative and hypnotic effects of alcohol may occur.

The investigators have presented evidence indicating that intravenous infusion of two grams per kilogram of alcohol produced depression of both serotonin and norepinephrine levels in the brain stem of the rabbit. The parallel depletion of both neurohormones persisted long after the alcohol had disappeared, an effect which is reminiscent of the action of reserpine. Chronic administration of alcohol for 7 days produced 50 percent decrease in both serotonin and norepinephrine levels in rabbit brain stem.

Scientists previously have suggested that reserpine works through releasing epinephrine and serotonin from storage depots in the brain and that its sedative effect results from the bombardment of central receptor sites by free serotonin. The significant similarity in effect on neurohormones which is produced by alcohol has now caused the investigators to theorize that the sedating and hypnotic effects of both drugs may be achieved through the same mechanism. If verified, this concept would provide a physiological explanation of the well-known mood depression of chronic alcoholics.
(NIMH grantees Drs. Deha Gursey and Robert Olson, University of Pittsburgh. Reported in Proceedings of the Society for Experimental Biology and Medicine.)

DELIRIUM TREMENS

The relationship between the serum magnesium level, delirium tremens, and other alcohol withdrawal symptoms are of vital importance in the treatment of chronic alcoholics. Work done thus far has shown that there is a decreased concentration of serum magnesium in patients with delirium tremens and alcoholic hallucinosis. Whether a serum magnesium deficit results from, or is the cause of, delirium, with its increased psychomotor agitation, disorientation, excessive perspiration, and hallucinosis, remains to be determined. However, the findings in a number of the delirium tremens patients who were studied during convalescence showed that the value of serum magnesium increased in each case without the use of magnesium in the therapy, thus failing to substantiate the hypothesis that a magnesium deficiency exists in alcoholic patients who have consumed copious amounts of alcoholic beverages and who have had a poor or restricted diet. (NIMH grantees J. Mendelson, D. Wexler, P. Kubzansky, H. Leiderman, and P. Solomon, Massachusetts General Hospital, Boston City Hospital, and Harvard Medical School, Boston. Reported in the Journal of Nervous and Mental Disease.)

ETIOLOGY OF ALCOHOLISM

Findings on the genesis of alcoholism have been contributed by a study in which members of the Department of Sociology at Stanford University investigated data which had been gathered on a group of male individuals, both before and after the onset of alcoholism in any members of the group. A longitudinal social study was made of the physiological, psychological, and social characteristics of a group of boys and their families, using a sample of 255 urban, relatively lower-class boys who were participants in the Cambridge-Somerville Youth Project. Originally selected and observed in the 1930's, these boys were then traced to adulthood. Twenty-nine of the subjects became alcoholics; the great majority did not. By statistically comparing those who became alcoholic with those who did not, the authors distinguished certain psychological, personality, and family traits that set the alcoholics apart as a distinctive human type in their childhood, long before the onset of their disorder. The major findings indicate that the alcoholic had undergone a variety of frustrating experiences. He was more likely to have suffered from various neurological disorders, and more likely to have been raised in a family disrupted by a high degree of conflict and basic disagreement.
(NIMH grantees W. McCord and J. McCord, Stanford University. Reported in Origin of Alcoholism, Stanford University Press.)

HIGHLIGHTS OF PROGRESS

IN RESEARCH ON NEUROLOGICAL

AND SENSORY DISORDERS

1960

Items of Interest on Program Developments and Research Studies

Conducted and Supported by the National Institute of Neurological

Diseases and Blindness

CENTRAL NERVOUS SYSTEM

NEURAMINIC ACID FRACTION IN CEREBROSPINAL FLUID RELATED TO NEUROLOGICAL DISORDERS

Studies of a neuraminic acid fraction in human cerebrospinal fluid have resulted in findings that variations in the fraction's concentration are related to some neurological disorders. Current reports indicate that the concentration levels are significantly lower in the ventricular (brain) fluid of patients with severe cerebral edema and are increased in cases of hydrocephalus due to brain obstruction.

The effect of certain neurological conditions on the activity of the new fraction has been reported in the Journal of Clinical Investigation by Drs. L. Lahut Uzman, Edgar A. Bering, and Charles E. Morris of the Harvard Medical School.

Interest in the neuraminic acid content of cerebrospinal fluid (CSF) is founded on the possibility that variations may reflect some aspects of central nervous metabolic activity. However, no pathological processes have been correlated with such variations until recently. Initial studies by Drs. Uzman and Bering showed that neuraminic acid in CSF is composed not only of a protein-bound fraction, but of a freely dialyzable fraction (capable of passing through membranes). Unlike the protein-bound fraction, the dialyzable form does not occur widely in human serum, and is therefore capable of reflecting metabolic changes.

The investigators tested levels of neuraminic acid concentration in 262 patients. From this group, several smaller groups of patients with specific neurological disorders were selected. Results of these tests showed that patients with noncommunicating hydrocephalus, where the outflow of fluid from the fourth ventricle was obstructed, had a high concentration of the dialyzable fraction in the brain. Lower concentrations were found when the hydrocephalus was not due to an obstruction. Similarly, fraction levels were very low in cases of spinal subarachnoid block when fluid was removed from below the site of the block. The authors suggest that this decrease may serve as a more sensitive index of physiologic block of CSF circulation than the current method of testing total protein elevation.

Patients with severe cerebral edema, regardless of cause, had a low proportion of the dialyzable fraction in the ventricular fluid. This may be due to metabolic disturbances and circulation restrictions caused by the edema, which interferes with the production of the fraction. The finding also lends support to the authors' suggestion that the new fraction is produced by metabolic activities within the brain. As expected, no correlation was found in the CSF content of either fraction in patients with chronic degenerative diseases of the nervous system.

In normal patients, the dialyzable fraction is most highly concentrated in the brain and decreases in the lower spinal region. In contrast, the protein-bound fraction is in lowest concentration in the ventricular fluid; under ideal conditions, a direct exponential relationship was found between the decrease of total protein and the increase of the dialyzable fraction. Further investigations, the authors suggest, may show this to be a useful method of approximating the neuraminic acid concentration in the brain in cases where ventricular fluid is not obtainable.

NEW TECHNIQUE SPEEDS NERVE IMPULSES BY SHORT-CIRCUITING INTERNAL RESISTANCE

The normal velocity of transmission of nerve impulses in the squid axon has been enormously increased through the use of low surface-resistance wires threaded along the interior of nerve fibers. By this technique, the nerve's longitudinal electrical resistance is short-circuited and conduction rates may become almost infinite over the short region occupied by the wire.

The technique also provides an example of an almost perfect "space clamp." An ideally space-clamped region is defined as one over which potential changes may occur, but where the potential is the same at every point at any instant. The new finding was reported in the Journal of Physiology by Dr. J. del Castillo,

formerly of the NINDB Electroencephalography Branch, and Dr. J. W. Moore of the NINDB Laboratory of Biophysics.

Previous experiments have shown that small changes in the speed of nerve impulse transmission are observed when the external longitudinal resistance is varied. However, much greater impulse transmission velocity was expected and found when the large internal resistance of the nerve was reduced by the insertion of a wire.

Giant motor-nerve fibers dissected from the squid were placed in a horizontal cell, and microelectrodes for measuring action potentials were inserted into the membrane. The transmission speed between these microelectrodes agreed with speeds reported by other studies. However, when an axial platinum wire electrode was inserted, a marked increase in conduction velocity resulted. In some cases, the time difference between action potentials recorded by the two microelectrode tips was reduced from a thousandth of a second to a few millionths of a second.

As was expected, the increase in the conduction velocity was inversely related to the surface resistance of the electrodes, so that with wires of resistances as low as 20 ohm centimeters, the conduction velocity was 230 times greater than normal over short lengths (1.5 cm.) of axon. The investigators also concluded that the space clamp achieved by using electrodes of this resistance approaches the ideal, since the action potential is generated almost simultaneously over the entire electrode.

STUDY SUGGESTS DIFFERENT NERVE IMPULSES MAY USE SAME PATHWAYS

Studies of a group of "common-sense" cells in the cat's spinal cord, which were found to respond to all skin stimuli, indicate that the traditional concept of anatomically separate sensory pathways may be modified to include systems which transmit information about more than one sensation. Reactions to skin stimuli in the cat and in man suggest that sensory messages may be transmitted along common pathways, and that the electrical discharge patterns of the central cells may determine the types of responses which occur.

Experiments which seem to confirm the theory of common-carrier pathways have been described in the A.M.A. Archives of Neurology by Dr. Patrick D. Wall and John R. Cronly-Dillon, NINDB grantees at the Massachusetts Institute of Technology. The investigators emphasize, however, that both specific and non-specific sensory pathways, as well as intermediate forms, may also exist.

These investigations were the result of the discovery of a second group of cells in the cat's spinal cord which receive messages

about pain, pressure, itch, and temperature from the skin. Intracellular recording of electrical activity indicated that many primary nerve fibers converge on this cell unit. The discharge patterns of these cells were found to vary with the type of stimulus applied to the skin.

When the cat's skin was damaged, or when itch-producing substances were applied, characteristic high-frequency discharge patterns were recorded from the central cells. The regularity of the patterns and of the nerve impulses transmitted indicated that the pattern probably resulted from the excitation of cells in the central group which had been partially inhibited. The investigators suggest that this patterned discharge may signal the responses which subsequently occur in other parts of the nervous system. However, it remains necessary to confirm that cells in the cat are in a sensory pathway rather than in a local reflex or cerebellar pathway.

In further experiments, light vibration of the skin was found to interfere with the response of the central cells to touch, damage, itching, and temperature. The authors suggest that the vibration probably interferes with the central inhibitory mechanism, especially as decreased electrical activity was also observed when the spinal cord was stimulated directly. Parallel results were observed in man, where light vibration was found to increase the threshold for the perception of touch, pain, and temperature to a statistically significant degree.

Results of this study indicate that the newly-discovered group of "common-sense" cells in the cat's spinal cord may have the ability to filter the impressions received from the skin. Thus, the filtering abilities of any cell group would determine the particular patterns of discharge that could be detected, thus enabling the cells to distinguish between the types of stimuli received.

The authors emphasize that only natural stimulation should be used to determine the nature of these filter systems, since the massive volleys from electrical stimulation do not give the cells a chance to demonstrate their ability to select, abstract, and modify the impulses.

NERVOUS SYSTEM DISORDER MAY BE ETIOLOGICAL FACTOR IN ORTHOSTATIC HYPOTENSION

Certain lesions of the nervous system, in association with an extreme drop in blood pressure when standing erect, have been observed in two patients having no systemic disease. Supported by other reports of similar cases, the study suggests that a primary "degenerative" nervous system disorder appears to be a recognizable clinical syndrome as a cause of at least some types

of orthostatic hypotension.

The investigators, Dr. G. Milton Shy, Medical Neurology Branch, and Dr. Glenn A. Drager, formerly with NINDB, found the most common initial symptoms of this condition to be impotence, incontinence, and loss of sweating; "dizziness" and blurred vision occurred on rising to a standing position followed later by fatigability and the sense of muscular weakness. The signs and symptoms observed in these patients were compatible with automatic nervous system involvement and a Parkinson-like syndrome. The report appears in the A.M.A. Archives of Neurology.

Any of the above signs and symptoms may be the presenting ones, according to the authors, and the hypotension may be a relatively late finding. Orthostatic hypotension may be present in some degree from the beginning of the illness, but usually is not detected until the patient has experienced extreme "dizziness" or syncopal attacks. The age of onset is usually in the fifties to seventies. The duration of the illness, from these cases as well as others cited in the literature, shows the disorder to be of relatively slow progression. The disorder appears to be more frequent in the male.

Within the past five years, the investigators have seen four clinically similar patients with orthostatic hypotension and signs and symptoms referable to the central nervous system. In the present study, pathological findings of one case are described in detail and do not appear to agree closely with pathological findings of hypoxia in the adult primate. A somewhat similar pathology has, however, been reported in hypoxic states in neonatal primates.

The authors conclude, therefore, that it is not possible, with evidence now available, to state whether the changes found in the central nervous system of this case precede the hypotension or are secondary to repeated episodes of hypoxia, but the difference in the sites of pathology suggests the former.

ORIGIN OF ELECTRICAL ACTIVITY IN NERVE CELLS AND MUSCLE FIBERS EXPLORED

A series of studies conducted by an NINDB investigator show that spontaneous, rhythmic electrical discharges occur in excitable tissues which receive no electrical energy from outside sources. The experiments suggest that the spontaneous activity may be generated by a disturbance of the intrinsic mechanism of individual nerve cells or muscle fibers. Additional studies of epileptic brain cells indicate that the same mechanism operates during seizures.

In determining the origins of electrical potentials in nerve cells in the brain, Dr. Chou-Luh Li, Surgical Neurology Branch, NINDB, found random small potentials similar to miniature end-plate potentials generated from motor nerve endings. Dr. Li summarized his studies of spontaneous rhythmic activity in lectures in the Department of Physiology, University of Chicago.

In three groups of experiments, glass electrodes were used to record intracellularly from single muscle fibers. In the first study, conducted on rats with intact motor nerve supplies, no spontaneous spike activity was observed. In subsequent experiments, where the sciatic nerve of the rats was severed, the electrical potential of muscle fibers was found to oscillate continuously, often setting off a series of rhythmic spike discharges. However, the spike activity did not affect the rhythmic oscillations. Similar results were obtained in a tissue culture study of isolated muscle fibers, which were also observed microscopically.

On the basis of these results, Dr. Li suggests that this spontaneous electrical activity must be related to periodic disturbances of metabolic or electrochemical factors, which are as yet unknown, within the denervated muscle cell. Similar observations were obtained on epileptic cells in the cerebral cortex, and were reported to the Symposium on Basic Mechanisms of Epileptic Discharges sponsored by the American Electroencephalographic Society.

BIOPHYSICAL STUDIES EXTEND KNOWLEDGE OF NERVE MEMBRANE PROPERTIES

Biophysical studies of electrical currents which cross the squid axon membrane confirm and extend the validity of theories of nerve impulse transmission which have been expressed mathematically by Hodgkin and Huxley. New data have resulted in the development of an equation which more accurately describes the characteristics of potassium ion flow through a membrane after it has been highly polarized, as well as under other electrical and environmental conditions.

A critical survey and evaluation of the Hodgkin-Huxley equations is being conducted by Drs. Kenneth S. Cole and John W. Moore, Laboratory of Biophysics, NINDB, who have reported the present study in the Biophysical Journal.

The investigators found that the original equations were valid when the membrane's electrical potential was initially at or below the resting level. When the membrane had been kept at an

abnormally high potential, however, the flow of potassium ions was delayed and these equations were not applicable.

Therefore, a new formula was developed to express accurately the delayed rise of the potassium current. This expression, although more complex, was found to apply under a variety of experimental conditions. Most important, both formulae were in close agreement in situations where the original work was applicable, indicating that the underlying assumptions are probably valid.

Experiments also supported the original theories that sodium and potassium ion currents across the squid axon membrane are independent of each other. In addition, they confirmed that the potassium current pattern is dependent on a single variable, defined by a first order differential equation.

The use of mathematical expressions greatly simplifies the interpretation of highly complex direct studies of ionic membrane currents. Although these processes cannot be investigated in higher animals or man, information obtained from the large axons of squid and several other invertebrates has been found applicable to vertebrates.

BRAIN AND CEREBRAL CORTEX

NINDB STUDY CHARTS BRAIN CONNECTIONS BETWEEN CEREBELLUM AND CEREBRUM

Studies have confirmed the presence of nerve pathways by which the cerebellum exerts a controlling influence over the motor functions of the cerebrum. Single shock stimulation of the hemispheres of the cerebellar cortex has resulted in consistent responses in the sensory and motor areas of the contralateral cerebrum, thus demonstrating a "circuit" between these parts of the brain.

Although cerebello-cerebral connections have been inferred from anatomical evidence, responses between these particular cerebellar areas and the cerebral cortex have not been demonstrated consistently. The results of a study of the connections by the evoked potential method is reported in Experimental Neurology by Dr. C. Murphy Combs and Sue V. Saxon of the Laboratory of Neuroanatomical Sciences, NINDB.

Data were obtained from 40 adult cats anesthetized with Nembutal or curare preparations. Silver ball electrodes were applied to the exposed cerebellar surface and a stimulus of from 7 to 12 volts was given. Responses from similar electrodes on the surface of the cerebrum were then recorded on a cathode-ray oscillograph.

From these tracings, the investigators found that the largest cortical responses could be obtained from the midsection of the anterior sigmoid gyrus--an S-shaped convolution of the motor cortex. Smaller potentials were consistently obtained from the lateral part of the posterior sigmoid gyrus. As expected, responses were much greater on the side of the cerebellar cortex opposite from the side stimulated. When responses from the same side were present, they were small, inconsistent, and occurred only in the anterior gyrus.

Early in the experiments it was observed that not all parts of the cerebellum were equally satisfactory for stimulating purposes. The most active parts of the sigmoid gyrus were then chosen as the recording point for the evoked potentials and various parts of the cerebellar surface were stimulated to determine the most efficacious stimulating points. Results of this experiment, contrary to a previous study, showed that the largest and most consistent results with the cerebellar hemispheres were obtained with low-voltage stimulation of the cerebellar area designated as crus I. The most active neocerebellar zone was found to be a longitudinal strip, bordered medially and laterally by unresponsive zones.

ELECTRON MICROSCOPIC STUDIES OF BRAIN SWELLING PROVIDE CLUES TO FLUID TRANSPORT

Excessive fluid within the brain, causing cerebral swelling, appears to be located solely within the clear glial cells, which may rupture in severe cases. The results of electron microscopic studies conducted by NINDB grantees also suggest that the clear glial cells, rather than intercellular space, may transport fluids in the normal brain.

The findings have resulted from detailed microscopic examinations of experimentally-produced swelling in the mouse brain and of swollen cerebral tissues which surround brain tumors in man. Results were reported in the American Journal of Pathology by Drs. R. M. Torack, R. D. Terry, and H. M. Zimmerman, Montefiore Hospital, New York.

Initial experiments concerned cerebral swelling which resulted from cold injury induced by the application of carbon dioxide ice to the mouse cerebrum. In these cases, electron microscopy revealed that the fluid accumulated solely within the cytoplasm of the glial cells. To confirm these findings, additional studies were undertaken of cerebral swelling induced by other means in the mouse, as well as of swollen human cerebral tissue.

Small amounts of alkyl tin compounds added to the diets of mice (12 to 32 parts per million) caused severe cerebral swelling, accompanied by progressive neurological involvement. When the affected tissues were examined with the light microscope, however, the excess fluid seemed to be located between the cells. This finding was then contradicted by electron microscopic examination, which showed that the fluid causing the swelling was localized exclusively within the clear glial cells, and that the intercellular space was unaltered.

Similar alterations were found in human tissue obtained from the soft, rather dry, swollen area surrounding several types of intracranial tumors. Again, enlarged clear glial cells were found, and there was no intercellular fluid accumulation.

In advanced stages of tin poisoning, some of the clear glial cell membranes appeared to rupture, although fluid did not seem to penetrate the intercellular space. Human brain tissue also revealed glial cell ruptures. The wetness of the whole brain which characterizes edema may result from the increased number of cell membranes which rupture as swelling becomes severe.

According to the investigators, these studies may also indicate that the intercellular cerebral areas have little or no importance in the normal transport of fluids, as previously thought. Since the clear glial cells are clearly affected in abnormal fluid accumulation, they apparently play an important role in the transport of essential fluids within the brain.

SUBCORTICAL BRAIN AREA MAY PLAY ROLE IN REGULATING MEMORY AND RECALL

A subcortical brain area known as the hippocampus may govern the execution of planned motor activity by "selecting" experiences for deposit as "memory traces" in higher brain centers. Furthermore, the hippocampus may aid in recalling the experiences for use in similar goal-directed situations.

Possible functions of the hippocampus are suggested by experiments which showed significant correlations between goal-directed behavior and recordings of brain wave activity in cats. Determinations of the structural arrangement of impulse pathways in and around the hippocampus seem to support the findings. The study was conducted by Drs. W. R. Adey and C. W. Dunlop, and C. E. Hendrix, NINDB grantees at the University of California, and is reported in the A.M.A. Archives of Neurology.

Electrodes permanently imbedded in selected areas of cats' brains recorded irregular, slow brain waves when the animals were first exposed to the test situation--a divided T-maze with a concealed food reward. However, when visual and auditory cues were provided, bursts of rhythmic slow waves occurred with each approach to the reward. As the animals learned (by successive trials), the rhythmic waves were rapidly restricted to the dorsal hippocampus and the adjacent entorhinal cortex. The rhythmic bursts persisted throughout the training period, and in late training were greatest in the entorhinal area.

Since the regular bursts occurred only shortly before and during the animal's approach to the goal and were not correlated with head and body movements, the authors suggest that the patterns are related to recall rather than to an orienting reflex. In addition, the specific burst activity disappeared when cues were not provided, but rapidly reappeared when the animal was retrained.

Electronic computers were then used to determine the sequence of brain wave activities in hippocampal and entorhinal areas. In untrained animals, impulses were found to travel from the hippocampus to the entorhinal cortex, as suggested by classical anatomical studies. In trained animals an opposite pathway was found and the hippocampus was activated by impulses from the entorhinal cortex. These results were consistent with the correlations in the previous experiments, and seem to provide structural evidence for a functional "way station" for screening incoming stimuli and for recalling memory traces.

ELECTRON MICROSCOPE STUDIES CLARIFY STRUCTURE OF SECRETORY BRAIN NEURONS

The neurosecretory system, consisting of nerve cells which secrete droplets that flow through axons to specialized organs, has been greatly clarified by electron microscope studies conducted by an NINDB investigator. For the first time, the fine structure and secretory mechanism of these cells in the brain of the goldfish has been observed. The neurosecretory cells, which are thought to supply hormones or their precursors to the pituitary body, are also found in higher vertebrates, including man.

Within the nucleus of the preoptic region of the goldfish, clusters of closely packed secretory nerve cells were found. Micrographs of individual cells showed a central zone which included multivesicular bodies and membraneous elements typical of the Golgi complex. Close by, numerous small, dense, spherical droplets enclosed in individual membranes were identified as the neurosecretory granules. Two sizes of droplets were found, 0.1 micron

and 1 micron or larger, and no intermediate stage between the types was seen.

Further observations indicated that the small droplets arose primarily from the Golgi complex, whereas the larger droplets originated by gradual transformation of the multivesicular bodies. However, only the small droplet form has been found in the nerve fiber leading to the pituitary body, indicating that these granules transport hormones or their precursors. The large droplets may be concerned with such other functions as intracellular metabolism.

Results of the study were presented by Dr. Sanford L. Palay, Laboratory of Neuroanatomical Sciences, NINDB, at the European Regional Conference on Electron Microscopy, Delft, Netherlands.

"DRINKING" BRAIN CELLS PROVIDE CLUES TO PROTEIN METABOLISM AND TRANSPORT

Some scientists now think that certain brain cells may ingest proteins and other soluble metabolites in much the same way that amoeba surrounds and entraps its food by the movement of pseudopodia. This process, known as pinocytosis or " drinking" by the cells, has been observed in the living cells grown in tissue culture using phase-contrast optics. Otherwise, pinocytosis can be studied by tracing inclusions of protein in the cells which were made visible by labeling with a fluorescent marker.

In the various cell types of the nervous tissue grown in tissue culture, pinocytotic activity (as seen by formation of clear vacuoles in the phase-contrast optics) was closely correlated with the uptake of fluorescent proteins and was characteristic for the individual cell type. This correlation suggests that the cellular uptake of protein might take place by the mechanism of pinocytosis.

Strikingly similar protein uptake by the glial (glue) cells was observed in the area of edema surrounding brain injury in animals into whose blood stream or subarachnoid space "marked proteins" had been introduced. Thus, although pinocytosis cannot be observed directly in cells in the living brain, this similarity in appearance of protein inclusions in cells grown in tissue culture to those seen in the brain suggest that pinocytotic mechanism may be active in the living brain at least under certain conditions.

This application of a technique using fluorescent proteins emphasizes the importance of the glia as a metabolic intermediary and a transmitter of substances in the brain tissue, as well as provides a technique for future investigations concerning the

problems of permeability of the cerebral blood vessels to proteins. The study was conducted by Drs. Igor Klatzo and Jaime Miquel of the Surgical Neurology Branch, NINDB, and was reported at the meeting of the American Association of Neuropathology.

CHRONIC NEUROLOGICAL DISORDERS OF INFANCY AND CHILDHOOD

"MAPLE SYRUP" DISEASE, A CEREBRAL DEGENERATIVE CONDITION DESCRIBED

A cerebral degenerative condition with onset to tonic seizures within the first week of life, and a rapid progress to death or decerebrate stage is described in Neurology by Dr. John H. Menkes, NINDB grantee at the Neurological Institute, New York City.

Ten cases of the disease, characterized by the presence of an unusual urinary odor resembling that of maple syrup, affecting four unrelated families are reported. A significant factor in the report was that pathological examination on two cases showed no abnormalities.

While the amino acid pattern at birth was found to be normal, an increased excretion of leucine, isoleucine and valine was found during later stages of the illness. An accumulation of 3 keto acids (alpha-ketoisocaproic acid, alpha-ketoisovaleric acid, and alpha-keto-beta-methyl-n-valeric acid) together with the absence of other metabolites along the degradative pathway of leucine suggests that oxidative decarboxylation of the alpha-keto acids is blocked in this condition. The odor of maple syrup has been attributed to the lactide of alpha-hydroxy-butyric acid.

One of the above-mentioned acids was present in the urine of a patient's mother and unaffected sister. Upon isolation it was found to be an aromatic acid of unknown structure.

Various possibilities as to the specific cause for the metabolic arrest have been examined. It has been suggested that high tissue levels of alpha-ketoisocaproic acid inhibit the synthesis of cerebronic acids.

PSYCHOLOGICAL EFFECTS OF NEONATAL ASPHYXIA STUDIED IN YOUNG RHESUS MONKEYS

Behavioral changes that may result from lack of oxygen at birth have been described for the first time in a controlled group of young rhesus monkeys. Psychological tests of monkeys partially asphyxiated at birth, and of control animals delivered in a similar manner,

indicate that normal animals are more emotional and more responsive to their immediate environment than are asphyxial animals. The studies were conducted by Miss Sue V. Saxon of the NINDB Laboratory of Neuroanatomical Sciences, and the Field Station of Perinatal Physiology, University of Puerto Rico School of Medicine, and reported at a meeting of the United Cerebral Palsy Association, San Juan.

Since asphyxiation of newborn monkeys has been shown to produce neurological deficits similar to those found in some human infants, the effects of partial asphyxia on behavior and learning is currently of great interest. Previous studies of guinea pigs, conducted in the NINDB Laboratory of Neuroanatomical Sciences, indicate that nervous system damage can be directly correlated with behavioral changes.

Subjects for the primate study were six rhesus monkeys, asphyxiated by clamping of the placental cord before Caesarian delivery, and five normal controls. The range of asphyxiation times in the experimental group was from 11 to 16 minutes, with a mean time of 14 minutes. When the studies were initiated, all monkeys were between six and ten months of age.

In the first series of experiments, animals were placed individually in a soundproof chamber for ten minute periods weekly for 15 weeks. Behavior was recorded at ten second intervals and scores were based on the number of times the animal displayed emotionality, locomotion, or contact with any of the five stimulus objects in the chamber. The emotionality score was based on previously determined criteria of emotional behavior such as sucking, convulsive jerking, rocking, and crouching.

Results of this study indicated that the asphyxial subjects locomoted significantly more, tended to contact objects more, and were significantly less emotional than the normal subjects. However, near the end of the series, locomotion scores in normal monkeys increased, and contact scores in the normal group exceeded the experimental. According to the investigator, the greater emotionality in the normal group accounted for these differences, which tended to level off as normal monkeys became adapted to the observational situation.

A follow-up study of the same monkeys at ages 10 to 14 months partially confirmed these assumptions, since no statistically significant differences between groups in frequency of locomotion or in stimulus contact were found. Surprisingly, however, normal animals continued to be more emotional than were the asphyxial animals. In this study, each animal was observed for three minutes four times a day for eight days.

The use of four stimulus conditions, ranging from an empty chamber to a complex condition (a toy bug) did not influence behavior patterns. For all of the conditions, it was found that normal animals locomoted slightly less and contacted the stimulus more often. No one emotional behavior was characteristic of any one animal or group.

In general, Miss Saxon concludes, the activity most characteristic of the animals deprived of oxygen was random or undirected locomotion. In addition, these animals did not appear to be as responsive or directed by their immediate surroundings as were the normal monkeys.

MATERNAL DIABETES SHOWN TO CAUSE INFANT ABNORMALITIES

Maternal diabetes has been further verified as one of the known conditions which may cause mental retardation and cerebral palsy in a small percentage of children. A comparative study by NINDB investigators shows that abnormalities in infants born to diabetic mothers occur about eight times more frequently than in a matched group of normal control mothers. In addition, perinatal mortality rates in diabetic mothers were found to be three times higher than in the control sample.

A detailed study, comparing the results of 235 pregnancies in diabetic and prediabetic mothers with 249 normal pregnancies, has been reported by Drs. Anatole Dekaban and Robert Baird, Section on Developmental Neurology, NINDB. The results of the study appear in the Journal of Pediatrics.

Forty-eight diabetic mothers were selected for the study from area hospitals. All were under 43 years of age, and had a history of at least two pregnancies after the diagnosis of diabetes. Normal control mothers, selected from the same hospital, were matched with the diabetic patients according to race, age, number of pregnancies, and social status. All mothers were carefully interviewed by the investigators and most of the offspring were personally examined. Children with abnormal findings were hospitalized for examination.

Although the absolute number of surviving infants diagnosed as abnormal was relatively small, abnormalities were found to occur eight times as often among infants of diabetic mothers. The abnormalities in these children included mental deficiency, congenital malformation, birth injury, and epilepsy. Total perinatal loss in the sample of diabetic mothers was 49.5 percent--three times higher than the corresponding rate in normal controls. In 78 prediabetic pregnancies (before diabetes was diagnosed), perinatal loss was 26.0 percent, midway between rates for diabetic and normal control mothers.

A statistical analysis of possible responsible factors showed significant correlations between outcome of pregnancy and the control of the diabetes at delivery, type of delivery, and the infant's condition at birth. The severity of maternal diabetes and the occurrence of complications during pregnancy were not statistically significant.

In the second phase of this study, a detailed analysis was made of the clinical abnormalities and pathological lesions found in the offspring of these diabetic mothers and controls. Autopsies were performed on the 18 stillborn infants and on 11 of the 13 neonatal deaths (occurring up to four weeks after birth). Follow-up studies of the six surviving abnormal children were made.

Results of postmortem examinations showed that the commonest cause of neonatal death was hyaline membrane disease, that is, the formation of abnormal membranes in the air passage. With one exception, congenital malformations found among the infants could be attributed to embryonic disturbances prior to the tenth week of gestation.

According to Dr. Dekaban, the increased incidence of birth injuries may be related to the generally oversized babies of diabetic mothers delivered at term. However, the precise etiology of mental deficiency in some children is not entirely clear and requires further study.

NEW NERVOUS SYSTEM DISORDER MAY BE DUE TO INHERITED METABOLIC DEFECT

A cerebral degenerative disorder, presumably due to an error of intermediary metabolism, has been described in five boys in two generations of the same family. Distinguishing features of the new syndrome are peculiar stubby white hair, early and severe growth retardation, and severe neurological impairment, beginning soon after birth and progressing to decerebration and death. Although the symptoms and familial nature of the disorder suggest it is of metabolic origin, no specific biochemical abnormalities have yet been demonstrated.

Recent studies have shown that certain types of mental retardation or cerebral degeneration are caused by inborn errors of metabolism, although the actual extent of the problem is unknown. The recognition and identification of metabolic defects which occur with retardation is at present a promising and important field of investigation. The present study was reported at the American Academy of Neurology meeting by Drs. John Menkes and Milton Alter (formerly of NINDB), Neurological Institute, New York, and Drs. Gerd Steigleder and David R. Weakley of the Columbia-Presbyterian Medical Center, New York. The investigation was supported by grants from the NINDB, NIAMD, and DGMS.

Two patients exhibiting the syndrome were examined by the investigators and data on three other cases were obtained from medical records and interviews. From the family history, it was determined that transmission is probably by a sex-linked recessive gene. A review of the literature confirmed that the disorder represents a clinical syndrome not previously described.

Microscopic examination showed that the stubby, coarse, white hair found in all the children was twisted, beaded, and split at regular intervals. Although cases with similar hair defects and mental retardation have been reported, none has occurred with a fatal prognosis. Additional histologic studies showed normal skin, hair follicles, and sebaceous glands.

Impairment in weight gain, severe mental retardation, and focal or generalized convulsions also occurred in all patients, and several had skeletal anomalies. Post-mortem examination, performed in two cases, revealed nerve cell loss in the cerebral and cerebellar cortex, and astrocytosis and cystic degeneration of the cerebral white matter.

Extensive biochemical studies of urine, cerebrospinal fluid, and blood were conducted to detect possible metabolic defects. However, all tests were within normal limits and no abnormal metabolites or altered amounts of normal metabolites were found. As in similar conditions where metabolic defects are suspected but cannot be defined, further study with new and improved techniques may eventually prove successful.

STUDY SHOWS INCREASE IN INFANT DEATHS FROM HYALINE MEMBRANE DISEASE

A comparative study conducted at Johns Hopkins Hospital suggests that a recent increase in premature infant deaths from hyaline membrane disease may be related to lowered oxygen concentrations in incubators.

The incidence of fatal hyaline membrane disease in liveborn babies weighing under 5½ pounds at birth was compared for two 5-year periods, 1944-48, and 1954-58, by Drs. Mary Ellen Avery and Ella H. Oppenheimer, NINDB special trainees at Johns Hopkins University School of Medicine. Their results were reported in the Journal of Pediatrics.

An important difference between the two test periods was the change in oxygen concentration used in incubators. In 1954-48, oxygen levels were greatly reduced since high concentrations were found to cause retrolental fibroplasia, a disease which causes blindness. During this period, the incidence of fatal hyaline membrane disease was 38.3 percent of autopsies, as compared to 24.3 percent in 1944-48, in liveborn infants weighing over 1,000 grams (2 lb.3 oz.) who lived more than 30 minutes and less than six days. The incidence of the disorder for all premature infant deaths, however, increased from 21.9 percent of autopsies to 26.2 percent.

The investigators found that the disorder was rare in babies who weighed under 1,000 grams, possibly because of the structure of the very immature lung. Fatalities from the disease were most frequent in infants weighing between 1,000 and 1,500 grams. Increases in birth weights over 1,500 grams (3 lb. 5 oz.) were associated with a marked reduction in disease incidence, which emphasizes the importance of prematurity in fatal hyaline membrane disease.

The study indicates, according to the authors, that some infants with respiratory distress may need more oxygen than they have been receiving. However, new medical techniques which allow more premature live births may also account for the increase in mortality from this disorder. In addition, the use of analgesia and anesthesia in childbirth may be a factor in the disorder and should be evaluated in further studies.

FREQUENT EPILEPTIC ATTACKS IN INFANTS MAY CAUSE MENTAL RETARDATION

Comprehensive studies by an NINDB investigator of infants suffering from frequent daily seizures have led to the hypothesis that the attacks themselves may cause retardation of development in a certain category of infants. These babies are usually under one year of age; their attacks are brief but very numerous, amounting to 30-100 or more a day; and clinical and laboratory examinations fail to reveal evidence of organic brain lesions. The last condition excludes children with brain damage in whom both epileptic attacks and retardation are caused by a common denominator--that is, some sort of cerebral damage.

Results of the preliminary study were reported in the AMA Journal of Diseases of Children by Dr. Anatole Dekaban, Section on Developmental Neurology, Surgical Neurology Branch, NINDB.

All of the six infants studied had developed normally until the onset of the frequent, but usually minor, epileptic attacks, which had begun before six months of age. Extensive examinations failed to show any physical or biochemical basis for the seizures, but brain wave recordings showed abnormal electrical activity in all. These and other findings suggested that idiopathic epilepsy may occur in early infancy.

A few weeks after the onset of seizures, these children displayed gradual slowing of motor and mental development, which usually was more pronounced when the attacks were most frequent. When the seizures were controlled, however, a striking but gradual improvement in development followed. The degree of improvement seemed to be related to the age of the patient, i.e., the younger the infant when seizures were stopped or diminished, the better was the prospect for his normal development.

Dr. Dekaban speculates that seizures may slow the development of initially-normal infants by hampering alertness and natural ability to respond to and learn from environment. Lost learning opportunities at certain critical ages may never be fully regained. A need for a study of greater scope in this field is stressed.

ELECTROENCEPHALOGRAPHIC PATTERNS RELATED TO INTELLECTUAL ABILITY IN BRAIN DAMAGED PATIENTS

A definite relationship between electroencephalographic patterns of brain damaged patients and the distribution of the patients' scores on the Wechsler-Bellevue intelligence scale has been demonstrated by an NINDB grantee.

In tests of intellectual ability in 185 patients with verified brain damage, the electroencephalographic pattern of left-sided brain damage was related to lower verbal abilities, while damage to the right hemisphere was associated with lowered scores on performance tests. In patients with generalized brain damage, no striking differences between verbal and performance scores were found.

A comparative study of four groups of patients, classified according to electroencephalographic criteria, was published in Neurology by Hallgrim Kløve of the Department of Neurology, Indiana University School of Medicine.

Electroencephalograms (recordings of brain waves) were administered to patients referred to the Indiana University Medical Center for neuropsychologic examinations. For the purpose of the study, patients were divided into four groups, i.e., those whose electroencephalograms showed maximal abnormal activity over the right hemisphere (group I), over the left hemisphere (group II), over both hemispheres (group III), and those with brain damage but with normal electroencephalograms (group IV). Before the groups were composed, the Wechsler-Bellevue Scale of Adult Intelligence and the Halstead neuropsychologic battery were administered to each patient.

Comparisons of Wechsler-Bellevue scores of groups I and II showed significant differences on both the performance and verbal weighted score means and on the individual subtests. Patients in group I were clearly impaired in performance areas and group II showed consistent impairment in verbal areas. However, no significant differences between performance and verbal scales were found in patients in groups III and IV, although the latter group, where electroencephalograms were normal, showed an absolute intelligence test level that was much superior to the other groups. Therefore, the author suggests, Wechsler-Bellevue results are not likely to

reflect impairment in brain damaged patients with normal electroencephalograms.

Halstead's impairment index, however, failed to show any significant differences between the groups, and the indexes were found in all cases to be within the range characteristic of groups with established brain damage. Although the study was not specifically designed to evaluate Halstead's impairment index, these findings may indicate that it may be even more sensitive to brain damage than the Wechsler-Bellevue scale and the electroencephalogram.

From the results of the present study, it appears that lateralization of a brain lesion may be determined through analysis of the Wechsler-Bellevue subtest pattern. These inferences, however, are valid only if the presence of brain damage has been established and if patients are not substantially different from those used in the study.

INVOLUNTARY MOVEMENTS

COURSE AND PROGNOSIS OF ACUTE CEREBELLAR ATAXIA STUDIED IN CHILDREN

New criteria for predicting the course and prognosis of acute cerebellar ataxia in children have been presented in Neurology by NINDB grantees Stuart Weiss and Sidney Carter of the Neurological Institute, New York City.

Studies of documented cases followed for periods of nine months to 17 years indicate that contrary to previous opinions, patients afflicted with this syndrome do not always recover completely. In addition, the report suggests that severe initial manifestations may result in slow recovery.

Although the clinical syndrome of acute cerebellar ataxia has been well documented, the few follow-up studies to date have been of short duration and have assumed a benign prognosis. Heretofore, the clinical picture has been described as of sudden onset, often shortly after a nonspecific infectious disease, with severe gait ataxia, and complete recovery within a few months.

In this study, records of children treated for acute ataxia at Columbia-Presbyterian Medical Center were reviewed and a study sample of 18 cases selected. Of these patients, 10 recovered completely within a six-month period after onset of illness. However, the remaining eight patients, affected with apparently the same illness, recovered more slowly, and, in some cases, incompletely.

Six of the patients--one-third of the series--showed persistent neurological deficits including gait disturbance, truncal tremor, ataxia of extremity movement, abnormal eye movement, delayed and impaired speech, and other symptoms indicative of mental retardation during follow-up periods ranging from nine months to six and one-half years. Impairment of speech and intellect, the authors suggest, may be due to a subtle type of cerebral involvement not detected initially.

Many factors were evaluated in all patients to determine whether the prognosis could be predicted. It was found that age at onset, race, sex, and presence of early symptoms were of no significance. The cerebrospinal fluid examination and other laboratory data obtained on initial evaluation proved of no value. However, the investigators found a definite correlation between the severity of the initial manifestations of the illness and a poor prognosis for recovery.

New knowledge relating to the possible etiology of acute cerebellar ataxia may also result from these studies. The prolonged course of the disease in one-third of the cases studied indicates that toxins, allergy, or viruses may not be causative factors. The early age at onset and the lack of new neurological manifestations over the long follow-up period suggest that multiple sclerosis or progressive demyelinating disease are probably not involved.

NEW MEASURING DEVICE EVALUATES PARKINSON THERAPY

A new "dynamic" measuring device for evaluating therapy and extent of disability in patients with Parkinson's disease has been developed by an NINDB grantee at the University of Minnesota Hospital, Minneapolis. The results of preliminary tests by Dr. David D. Webster show that the technique accurately measures the effects of medication and stereotactic surgery on rigidity, strength, and tremor in Parkinson patients. The study is reported in Neurology.

The technique utilizes a powerful yet highly sensitive turntable, to which the patient's forearm is rigidly coupled by a split plaster cast. The arm is then alternately flexed and extended horizontally through an arc of 100° at various speeds. Energy required to move the forearm is automatically calculated for different rotational speeds during both active and passive motion. Unlike previous methods, the machine minimizes extraneous stimuli which may precipitate tremor in Parkinson patients.

By means of a pen writer, the system records tremors as fine as 10 grams at maximum sensitivity. In tests of strength, patients are instructed to voluntarily resist the turntable motion, and resistances as high as 90 foot-pounds may be measured. Pens also record the position of the forearm and the net work done by the turntable in moving the patient's forearm through each cycle of passive motion.

Comparisons of the test data for passive motion showed that the expenditure of energy recorded at the end of each cycle was nearly proportional to the extent of muscle rigidity. Results also showed that the maximum force required to initiate movement in the arm of a patient moderately afflicted with Parkinson's disease was twice that required to move the normal arm. In addition, the energy

expended by the apparatus in moving the afflicted arm through the cycle was 10 times that of the normal. This appears to be an accurate method of measuring rigidity, since elastic muscle reactions are cancelled out after each cycle.

In tests of muscle reaction to activity, a flashlight was placed in the patient's free hand, and he was instructed to follow the beam of a second flashlight directed onto a screen by the operator. Although normal individuals showed no increased rigidity, this simple test was found to "precipitate" rigidity in patients with Parkinson's disease. Both the active and passive motion tests were used to evaluate the effects of medications and surgery on rigidity, strength, and tremor.

Responses to medication were studied by this method in several patients with varying degrees of tremor and rigidity. Because some drugs or combinations of drugs are more helpful to some patients than others, the effects of medication on the individual patient must be constantly evaluated. This technique made it possible to quickly indicate the degree of improvement or regression in response to various therapies.

At present, this technique is being used as part of a study of the effect of stereotactic surgery in Parkinson's disease. In the cases described, significant reductions in symptoms were recorded by the apparatus and clinical improvement was simultaneously observed.

STUDIES SUGGEST BASIS FOR SUCCESS OF BRAIN SURGERY IN PARKINSON'S DISEASE

Experiments which may explain why brain surgery relieves the symptoms of Parkinson's Disease suggest that muscle rigidity and weakness may be caused by inhibition of nerve circuits between the spinal cord and bundles of muscle fibers (spindles), which promote steady voluntary contraction. Surgically-produced lesions of the thalamus of the brain may reduce the inhibitory effect, thus restoring functional and balanced nerve activity.

Electrical stimulation of an area of the thalamus of the cat, investigators found, consistently inhibited afferent discharges from the muscle spindle to the spinal cord. It appears, the authors suggest, that the central nerve pathways which promote the action of muscle spindles are similarly inhibited in Parkinsonism. When the cat's muscle was stretched, the inhibitory effect was diminished; and destruction of the motor cortex abolished it entirely.

Previous studies have suggested that the activity of nerve circuits supplying the main muscle increases when spindle discharges decrease. Therefore, the rigidity and weakness of Parkinsonism

may be the result of an altered balance between the two spinal cord circuits which innervate muscles. If this theory is true, surgical lesions of the thalamus reduce the inhibitory input to the muscle spindle and balance the activity of nerve circuits, with a corresponding improvement of rigidity and weakness.

The study was reported in the A.M.A. Archives of Neurology by Drs. Jack Stern, NINDB Post-Doctoral Fellow, and Arthur A. Ward, Jr., NINDB grantee, of the University of Washington School of Medicine, Seattle.

NEUROMUSCULAR DISORDERS

STUDY YIELDS CLUES TO CAUSE OF FAMILIAL PERIODIC PARALYSIS

By correlating the results of multidisciplinary investigations, NINDB scientists have clarified the nature and possible cause of a baffling neurological disorder known as familial periodic paralysis. New evidence indicates that muscle action may be disrupted by increased accumulation of fluid within the endoplasmic reticulum of muscle cells. The fluid increase may be caused by defective glycogen metabolism in muscle, resulting in an altered ionic balance.

Intensive clinical, biochemical, electron microscopic, and electrophysical studies of familial periodic paralysis have been conducted by Drs. G. Milton Shy, Theodor Wanko, Peter T. Rowley, and A. G. Engel, Clinical Investigations Unit, NINDB. A significant result is the demonstration that muscle potassium and sodium are not significantly elevated during attacks and that muscle becomes electrically unexcitable. Recording of the membrane potential by microelectrodes within the muscle cell also refutes the theory that paralysis in this disease is due to hyperpolarization by potassium accumulation.

Samples of muscle tissue obtained during attacks indicated that increased fluid was contained in large vacuoles within a specific conductive network of the muscle cell, the endoplasmic reticulum. Inside the vacuoles, granules which took up certain stains were found. This and other evidence suggests that the granules may represent the accumulation of abnormal glycogen breakdown products, which cause an influx of electrolytes and water into muscle cells to maintain ion balance.

The investigators reconfirmed that the administration of glucose, glucose and insulin, and epinepherine predisposed to attacks of weakness or paralysis. Administration and measurement of aldosterone, a potassium diuretic and sodium retention steroid, indicated that abnormal metabolism of this hormone is probably not a cause of the disease, as has been suggested. For unknown reasons, however, an excess sodium intake precipitated attacks.

Although familial periodic paralysis has been associated with hyperthyroidism, increased or decreased levels of thyroid stimulating hormone produced no adverse effects. However, low basal metabolism rates were found to aggravate symptoms, and elevated basal metabolism was associated with relief of symptoms, confirming altered metabolism of muscle as a primary defect in this unusual disorder.

The studies were presented at the scientific session of the Centenary of the National Hospital, Queen Square, London.

NEW DRUGS FROM DAFFODILS SHOW PROMISE IN TREATMENT OF MYASTHENIA GRAVIS

Pharmacological studies to evaluate galanthamine, a drug reported by Russians to relieve some neuromuscular disorders, have resulted in the identification of a promising new series of drugs, which are chemically similar but more potent than galanthamine. Studies in animals strongly suggest that the newly-synthesized compounds may be effective in treating myasthenia gravis, and some are more potent than drugs now used for this disorder. Preliminary clinical trials of one of the compounds have recently been initiated.

The activity of galanthamine, its derivatives, and other chemically-similar compounds in increasing muscle contraction and inhibiting cholinesterase has been evaluated by Dr. R. L. Irwin, Medical Neurology Branch, NINDB. These compounds were also compared with drugs now in use to treat myasthenia gravis. Although the cause of myasthenia gravis is not known, drugs which inhibit cholinesterase at the nerve-muscle junction usually produce striking relief of symptoms.

Initial studies showed that the drug reported by Russian scientists, galanthamine hydrobromide, was significantly less potent than the quaternary form of the compound, galanthamine methiodide. However, the latter drug was still not as effective in inhibiting cholinesterase as drugs now in clinical use, such as neostigmin.

Subsequently, the derivatives of lycoramine, which are structurally similar to galanthamine, were tested on muscle for the first time. Two of these derivatives were found to be more potent than neostigmin, although their toxicity was greater. A third compound was less toxic than neostigmin, yet was more active than physostigmine. It also had a slower onset of action than the other derivatives and was absorbed after oral administration. At present, the third derivative seems to be the most promising for clinical trial.

Both the galanthamine and lycoramine compounds are particularly interesting because they differ chemically from drugs currently used to treat myasthenia gravis. In addition, they can be obtained

from the bulbs of plants in the Amaryllis group, such as the daffodil, which are readily available throughout the world. The specific compounds tested by Dr. Irwin include: galanthamine hydrobromide, galanthamine methyl iodide, lycoramine methyl iodide, deoxylycoramine methyl iodide, deoxydemethylycoramine methyl iodide, lycoramine acetate methyl iodide, neopine, and neopine methyl iodide.

The results of the studies were presented at the meeting of the American Society of Pharmacology and Experimental Therapeutics, Seattle.

PROGRESSIVE SKULL CHANGES OBSERVED IN HEREDITARY MUSCULAR DISORDER

An NINDB study has confirmed that "hyperostotic" skull changes are a variable finding in dystrophia myotonica, an inherited type of muscular dystrophy. Of eighteen patients with the disease, skull changes were observed in the X-rays of seventeen (94 percent) by Drs. G. Di Chiro and J. E. Caughey, Neuroradiology Branch, NINDB. Observations suggest that the changes are progressive and that they may be related to increased growth hormone activity.

Four types of radiographic changes in the skull vault were defined: thickened calvaria throughout, thickened calvaria in the frontal region, irregular bulgings of newly-formed bone in the frontal region (hyperostosis frontalis interna), and thickened calvaria throughout with hyperostosis frontalis interna. Changes of the vault appeared with equal frequency in males and females, although previous studies have shown that cranial hyperostosis occurs almost exclusively in women.

The most marked skull changes were found in patients afflicted with the disease the longest. In addition, the degree of change was greatest in eight patients with confirmed gonadal failure, suggesting that the unrestrained activity of growth hormone may be a cause of the progressive skull alterations. The study appears in Acta Radiologica.

MULTIPLE SCLEROSIS

ALLERGIC ENCEPHALOMYELITIS QUANTITATIVE INDEX DEVELOPED

A quantitative index correlating the clinical signs and severity of lipemia with histologic lesions has been developed to aid in assaying encephalitogenic activity of brain extracts.

By studying accumulated data on a large number of animals, injected with brain or active chemical fractions of nervous tissue, Dr. Ellsworth Alvord, NINDB grantee at Baylor University College of Medicine and Dr. Marian Kies, Research Branch, National Institute

of Mental Health, Bethesda, developed the index. A description of their work appears in the Journal of Neuropathology and Experimental Neurology.

Many attempts have been made to demonstrate the activity of various chemical fractions of brain in producing encephalomyelitis when injected parenterally with adjuvants. Although decreasing doses to determine the threshold or 50 percent effective dose have been used for whole brain, only one similar study has been made with various fractions of brain in a search for the causative factor or factors.

Approximately 1,000 healthy adult male guinea pigs were used for the study. Following an initial observation for 2 weeks, groups of 10 animals were injected intra-dermally with vaccines containing various amounts of brain or brain-extracts in Freund's adjuvant. After 30 days, survivors were anesthetized, blood collected, and the animals killed by perfusion intracardially. Clinically positive animals were sacrificed when they had developed unquestionable signs of the disease. The brain and spinal cord were removed for neuropathologic examination. Clinical signs, degree of lipemia, and degree of histologic changes were coded according to severity. It was observed that lipemia practically did not occur except with the combination of severe clinical and histologic changes. Correlations between these 3 factors were then made, first considering all of the possible subdivisions, and subsequently combining various subdivisions which did not appear to be significantly different from each other.

By plotting the mean numerical index of the severity of the experimental disease against the weight of material injected, a curve was obtained demonstrating the severity of the disease produced by different doses of the particular material. The dose that produced 50 percent of the maximal severity of the disease was termed ED_{50} (50 percent effective dose) and could be used as a single index of the effectiveness of the particular substance injected. A close approximation to the ED_{50} was made by using the dose that produced a disease index of 4; this was chosen because the average maximum disease index was about 8 (on a scale ranging from 0 - 10). In this way whole guinea pig brain was compared with bovine spinal cord, cord acetone powder, lipid-free residue of cord, water soluble, 5% KCL soluble, and water insoluble residue of cord as to comparative activities in producing experimental allergic encephalomyelitis in guinea pigs. Of all of these, the 5 percent KCL was the most active material per unit weight. It was approximately 6 times as active as lyophilized guinea pig brain and 10 times as active as lyophilized bovine spinal cord. The assay is being used to study the isolation and purification of the compound or compounds in the central nervous system responsible for producing the disease.

"ALLERGIC" ENCEPHALOMYELITIS IN GUINEA PIGS SUPPRESSED WITH BRAIN EXTRACT

For the first time, experimental "allergic" encephalomyelitis induced in guinea pigs has been suppressed by administration of the same chemical component which most effectively produces the disease. Allergic encephalomyelitis in animals (EAE) is histologically similar to a number of demyelinating diseases in humans, and has been linked with such disorders as post-vaccinal and post-infectious encephalomyelitides, and acute multiple sclerosis.

The important discovery was the surprising result of experiments initiated to test skin reactions to encephalitogenic vaccines which are known to cause the disease in animals. The findings were reported by Drs. Cheng-Mei Shaw, Willson J. Fahlberg, and Ellsworth C. Alvord, Jr., NINDB grantees at Baylor University College of Medicine, and Dr. Marian Kies, Research Branch, National Institute of Mental Health, Bethesda, and appear in the Journal of Experimental Medicine.

Although prevention by pretreatments has been reported, suppression of EAE by treating animals previously injected with disease-producing vaccines has been demonstrated only once. In the similar study, whole brain rather than isolated fractions was used.

To induce the disease, the investigators prepared vaccines of whole homologous brain extract and killed tubercle bacilli in water-in-oil emulsion (Freund's adjuvant technique), which were injected into the skin of guinea pigs. Skin-test solutions of acid-extract of defatted guinea pig brain were then administered at intervals. Surprisingly, animals repeatedly skin-tested at 11, 15, 21, and 28 days after injection of vaccines did not develop significant clinical or histologic evidence of the disease. Further studies were then initiated to determine whether the suppression was real or a statistical artifact, and whether it was due to the tuberculin, to the brain extract, or to some non-specific stress reaction.

In conducting the study, 50 to 80 white male guinea pigs were injected with encephalitogenic vaccine, and sub-groups of 5 to 10 animals received skin-test solutions on predetermined days after initial injection. Each animal, including uninjected controls, was observed daily for evidence of disease. After 30 days, survivors were sacrificed and brain and spinal cord were examined. In addition, animals were scored according to a graded

"disease index" and an average disease index was obtained for each group for statistical analysis and comparison.

Although the investigators found no significant results when 0.5 mg. of acid-extract was given on the 11th and 15th days, there was almost complete suppression when five such injections were given on each of the 9th, 14th, and 21st days. Additionally, larger doses were found to produce more complete suppression of the disease, indicating a quantitative relation to the time of administration and the dose administered. A non-specific stress situation, produced by intra-dermal injections of 20 percent alcohol, did not affect the disease. Furthermore, the effects of tuberculin were statistically insignificant and inconsistent.

At present, the etiology and pathogenesis of "allergic" encephalomyelitis is unknown. However, some clue may be afforded by the finding that a more active extract is more effective, suggesting that the suppression is due to a specific desensitization, defection or neutralization of antibodies, or paralysis of antibody forming mechanisms. The absence of skin reactions in both injected animals and in controls, however, indicates that an immunologic process is not involved.

Further experiments on this complex disease reaction are necessary to determine if the suppression is due specifically to encephalitogenic extracts, as contrasted to non-encephalitogenic, and to elucidate the mechanism of the suppression.

GEOGRAPHIC VARIATION IN PREVALENCE OF MULTIPLE SCLEROSIS VERIFIED

An epidemiological survey conducted in two comparable, but widely separated, communities showed that the prevalence of multiple sclerosis is 2.4 times greater in Halifax County, Nova Scotia, than in Charleston County, South Carolina. The survey was conducted by a single team of investigators who used uniform screening, classification, and diagnostic procedures.

The two communities were selected for the survey because they are similar in population, size, area, medical facilities, and are Atlantic seaports. They differ, however, in racial composition, geographic latitude, and climate.

Identical pre-determined criteria were used to screen provisional cases, which were then classified by category. The same team of investigators personally examined most patients in both communities.

Results showed that prevalence rates per 100,000 inhabitants based on clinically acceptable cases, were 13.8 in Charleston and 32.3

in Halifax. For the white population alone, the prevalence was 1.7 times greater in Halifax, and no preponderance of cases was found to be characteristic of any ethnic group.

Further investigations revealed no factors which accounted for the prevalence differences. Age distribution of patients was similar in each community, as were clinical and genetic manifestations of the disease. The age-specific prevalence rates were higher in Halifax in almost every age group.

Drs. Milton Alter and Leonard T. Kurland, Epidemiology Branch, NINDB; Dr. R. S. Allison, Royal Victoria Hospital, Belfast, Northern Ireland; and Dr. O. Rhett Talbert, Medical College of South Carolina, Charleston, reported their findings in World Neurology. This new journal also contains a report of a related NINDB-supported study, co-authored by Dr. Kurland, which indicates that the prevalence of multiple sclerosis may be less in Japan than in North America or Western Europe.

PATTERNS OF NERVE DEMYELINIZATION DIRECTLY OBSERVED IN TISSUE CULTURE

A new approach to demyelinating disorders such as multiple sclerosis has been developed by NINDB grantees who have induced and studied nerve demyelinization in tissue culture. Serum from animals with experimental "allergic" encephalitis (a condition resembling certain human demyelinating diseases) was found to produce patterns of demyelinization in cultures of newborn rat cerebellum. No such reaction was obtained after treatment with blood serum from normal animals or with anti-kidney serum.

The serum is toxic only to brain tissue, as cultures prepared with liver, muscle, and peripheral nerve tissue did not react to it. The rapidity of the reaction indicated that the response is probably not due to an infectious process.

The response begins with pronounced swelling of the neuroglia cells and myelin sheaths. Often, fragments of myelin sheaths are extruded into surrounding areas. Both the fragments and the sheaths then rapidly disintegrate and are converted into small fat granules. Neuron cell bodies and axis cylinders are left intact, just as they are in demyelinating disease.

Possible further applications of the tissue culture model have been suggested by Drs. Murray B. Bornstein and Stanley H. Appel, NINDB grantees at Mount Sinai Hospital, New York, who reported the findings at the American Academy of Neurology meeting.

MECHANISM OF BIOSYNTHESIS OF LONG CHAIN FATTY ACIDS DESCRIBED

The detailed sequence of biochemical reactions for the formation of long chain fatty acids in the cells of living animals has been described for the first time by an NINDB investigator. Fatty acids combine with glycerine to form fats, and are essential components of myelin, the fatty nerve sheath which degenerates in such disorders as multiple sclerosis.

In studies with purified enzyme systems from liver and brain, Dr. Roscoe Brady, Laboratory of Neurochemistry, NINDB, has shown how acetic acid, a two-carbon unit, is converted to a 16-carbon chain fatty acid known as palmitic acid.

Early studies conducted by Dr. Brady and other scientists established that the presence of carbon dioxide was essential for fatty acid synthesis. Subsequent work by Dr. Brady indicated that the key intermediate in the biosynthesis is a derivative of malonic acid, known as malonyl coenzyme A.

As a result, Dr. Brady was able to synthesize malonyl coenzyme A by adding carbon dioxide to acetyl coenzyme A, a derivative of acetic acid. By labelling these compounds, he discovered that one molecule of acetyl coenzyme A and seven molecules of malonyl coenzyme A, combined with 14 atoms of hydrogen, are required to build a molecule of palmitic acid.

The steps in the synthesis are as follows: When acetyl coenzyme A combines (condenses), the newly added carbon dioxide is displaced. When two atoms of hydrogen are added, the resulting compound can condense with another molecule of malonyl coenzyme A. This process is repeated until the appropriate chain length is reached.

To identify the intermediates which participate in these reactions, the required enzyme systems were isolated and purified. Each step in the sequence of reactions leading to fatty acid synthesis was defined by radioactive labelling of synthetic intermediary compounds. This basic finding, which has been presented, will be reported in the Journal of Biological Chemistry, and may aid in the understanding of metabolic defects associated with diabetes and degenerative disorders of the nervous system. The study will also be described to the International Biochemical Congress (Symposium on Lipid Synthesis) in Moscow, U.S.S.R., in August 1961.

AMYOTROPHIC LATERAL SCLEROSIS

POSSIBLE CAUSE OF SPINAL CORD DISEASE DEMONSTRATED BY NINDB GRANTEE

An experimental disorder, similar to some human virus infections and degenerative disorders of the nervous system, has been produced in cats injected with 5-fluoroortic acid (FO), a pyrimidine analogue which was found to interfere with the production of nucleic acid in nerve fibers. These studies suggest that similar metabolic disturbances may occur in some diseases affecting the gray matter of the spinal cord, such as amyotrophic lateral sclerosis.

Functional and structural disturbances produced by FO in the nervous system, as well as preliminary biochemical studies of its action, have been reported in the A.M.A. Archives of Neurology by Dr. Harold Koenig, NINDB grantee at Northwestern University Medical School. Subsequent biochemical work was reported at the annual meeting of the American Academy of Neurology.

Intrathecal injection of FO in cats was found to produce two independent neurological disorders. The initial disorder, a neuronopathy, affected the neurons, or spinal gray matter; the second, which appeared several weeks later, was a leukomyelopathy, affecting the white substance of the spinal cord. Clinical signs of neuronal irritation, similar to those occurring in many progressive diseases of gray matter in man, appeared one or two days after injection, causing spontaneous contractions of muscle fibers, myoclonic

jerks, and augmented muscle tone and reflexes. If the neuronopathy was sufficiently severe, loss of neuronal function ensued, characterized by a flaccid paralysis with loss of reflexes and severe muscle atrophy.

Pathological changes were confined to the gray matter for several weeks, and consisted of a progressive depletion and disruption of the Nissl substance (protein bodies in the cytoplasm of nerve cells). According to Dr. Koenig, this was probably due to impaired biosynthesis of ribonucleic acid (RNA) in nerve cells. This was then confirmed by biochemical studies where radio-active carbon atoms were added to FO. Autoradiographic studies showed that the compound was effectively incorporated into the RNA of neurons and oligoglia--tissues which surround the nerve's myelin sheath. Derivatives of FO were found in the acid-soluble fraction and RNA of neural tissue, indicating that efficient anabolic conversion had taken place.

Therefore, says Dr. Koenig, FO probably causes neurological dysfunction by producing defective co-enzymes and abnormal RNA molecules, thus altering the normal mechanism of protein synthesis. Eventually, such interference brings about a depletion of RNA in the spinal cord.

After the second week of illness, a spongy degeneration of white matter in the spinal cord appeared, associated with more severe neurological changes. Dr. Koenig suggests that this is due to a blockage of DNA synthesis, with a resultant inhibition of cell division in the oligoglia. Defective proliferation of the oligoglia may play a similar role in the spongy degeneration of white matter which occurs in pernicious anemia.

Other pyrimidine analogues tested produced no notable neurological effects, initially verifying that the changes were caused by specific metabolic disturbances. Biochemical studies further clarified this theory, since labeled carbon studies showed the impairment to be due to conversions of FO which interfered with nucleic acid biosynthesis. The degree of protein inhibition was found to parallel closely the severity of the neuronopathy.

EPILEPSY

MECHANISMS UNDERLYING EXPERIMENTAL EPILEPTIC SEIZURES DESCRIBED

Repetitive, self-sustained electrical discharges from brain cells, very similar to those taking place during human epileptic seizures, may be caused by a slow process of cellular repolarization, which occurs after the cell membrane has been excessively depolaried by repeated electrical stimulation.

In an experimental approach to the problem of epilepsy, Dr. Paul Gerin, former guest worker in the Electroencephalography Branch, NINDB, systematically analyzed spike patterns elicited by repeated electrical stimulation of brain cells. An interpretation of the mechanisms which may cause afterdischarge, that is, self-sustained activity which follows repetitive electrical stimulation, has been published in the Archives italiennes de Biologie. After-discharge is very similar to spontaneous seizures recorded from the exposed cortex of epileptic patients, and is often used as a tool for experimental study of epileptic activity.

Electrical stimulation of varying strength, frequency, and duration was applied to the cerebral cortex of adult cats with bipolar silver electrodes. The activity of single cells during and after stimulation was recorded by means of tungsten microelectrodes and by larger electrodes on the surface of the cortex. Of a total of 2,500 afterdischarges elicited, 600 were accompanied by visible spike activity and were recorded on film. Detailed analysis was made of activity in 80 different cortical cells.

Repeated stimulation, Dr. Gerin found, was usually accompanied by progressive and characteristic changes in the size and frequency of spike activity elicited by each electric pulse. When these changes did not occur, the phenomenon of afterdischarge rarely developed. Changes included a tendency for units to discharge repetitively and progressive decreases in the amplitude of spikes until activity sometimes ceased entirely.

When the spike firing ceased entirely, self-sustained electrical activity (afterdischarge) usually occurred, characterized by small, high frequency spikes, which progressively increased in amplitude and decreased in their rate of firing. The self-sustained activity eventually ceased when the spikes returned to the pre-stimulation level of amplitude.

According to Dr. Gerin, spike changes during and after stimulation may be ascribed to changes in polarization levels in the cell membrane. Thus, during repeated stimulation, recovery of the resting polarity level cannot occur, and a progressive depolarization of the membrane results. When this depolarization exceeds a critical level, firing stops and, during the process of cell repolarization self-sustained activity develops. Although conducted on an experimental level, these studies suggest that this unusually slow process of repolarization might be the characteristic feature of "epileptic" neurons.

BRAIN WAVE PATTERN CORRELATED WITH THAT FOUND IN BRAIN TUMOR PATIENTS

An electroencephalographic (brain wave) pattern, which frequently occurs in patients with idiopathic petit mal epilepsy, has also been found, with statistically significant frequency,

in patients with brain tumors. Detailed findings of two cases where tumors were associated with this EEG pattern and an extensive review of similar cases indicate that the abnormality which produces the typical "petit mal" pattern may lie within the brain stem. Unlike many other types of epilepsy, seizures which show this pattern usually cannot be correlated with gross or microscopic lesions in the brain.

Among the most striking and typical in clinical EEG, these brain wave recordings are characterized by bilateral, symmetrical 3/second wave-and-spike complexes. Studies relating this pattern with pathological findings were reported by Dr. C. Ajmone-Marsan and Dr. W. R. Lewis, NINDB Electroencephalography Branch, at the Eastern EEG Association Meeting, Ste. Marguerite, Quebec, Canada.

In the two cases examined by the investigators, both EEGs showed the typical pattern, highly suggestive of petit mal epilepsy. In the first subject, a large metastasis of one cerebellar hemisphere was found, but the patient had no history of epileptic seizures. In a second case, the patient demonstrated petit mal, as well as temporal lobe epilepsy and generalized convulsion. Here, a large astrocytoma extending into the thalamus was removed from the right temporal lobe.

Because cases of brain tumors associated with bilateral 3/second wave-and-spike patterns are so rare, statistical evaluation was devised to assure that a valid relation existed between the two phenomena. The respective incidence of brain tumors and of the "petit mal" pattern were calculated for the general population. Results showed that if chance alone were operating, an association of the two factors would occur in 48.2 out of a million cases. However, data from three published reports totaling 1,071 brain tumors resulted in an "actual" frequency of 6 cases, as compared to an expected occurrence of .0517 case on the basis of chance. According to the investigators, this discrepancy is significant enough to warrant the conclusion that the phenomena are related.

The predominant extra-cortical, mid-line location of brain tumors in the cases examined as well as in all similar cases reported in the literature suggests that the brain stem plays a significant role in the production of this very characteristic, yet still mysterious, EEG pattern.

BRAIN WAVE PATTERN MAY BE A MEANS OF LOCATING EPILEPTIC LESIONS

A characteristic pattern of brain wave activity has been found by NINDB grantees to monitor the onset of both penicillin-induced and spontaneous epileptic seizures in man. Since this pattern, unlike

the isolated, inter-ictal spikes, is sharply localized to the epileptic focus, its detection should be useful for locating epileptic lesions during surgery.

The phenomenon of "afterdischarge" had been described in humans by Drs. Bruce L. Ralston (formerly of NINDB) and Christos A. Papatheodorou, NINDB grantees at Mount Sinai Hospital, N.Y. Their report appears in Electroencephalography and Clinical Neurophysiology. These findings confirm Dr. Ralston's previous experimental studies in cats and monkeys, which showed that full seizures developed from similar afterdischarges rather than from progressive increase of inter-ictal spike frequency.

Brain-wave recordings directly from the cortex of animals (electrocorticography) indicated that penicillin applied to the brain was capable of eliciting afterdischarge, followed by fully developed seizures. As a result, experiments were initiated to determine if similar activity existed in human convulsive disorders.

Since spontaneous seizures are rarely recorded during electrocorticography and electrically-induced seizures are of a somewhat different type, penicillin was also used as an activating agent in man, prior to cortical surgery. The investigators found that the rapid, low-voltage afterdischarge could be easily elicited, and that it also could progress to fully developed seizures. Although the frequency of occurrence of the phenomenon is not yet known, no seizures were seen to develop through a simple increase of spiking activity.

Examination of a patient with temporal lobe epilepsy confirmed that such afterdischarge is also a characteristic of spontaneous seizure activity. In this case, the afterdischarge pattern was observed to occur at the same time as the abdominal sensation (epigastric aura) which often precedes epileptic seizures.

According to the authors, the recognition of afterdischarge may be of practical importance, because unlike spike patterns, which are often projected widely from their original area, the afterdischarge tends to remain clearly localized to the epileptic focus. Thus, it should prove a valuable means of determining the primary epileptic focus for surgical removal. In addtion, these studies clarify the still unknown mechanisms of seizure formation in man by defining more precisely the changes which the electrical activity undergoes during the development of an epileptic seizure.

BRAIN'S WATER AND ELECTROLYTE BALANCE RELATED TO SEIZURE SUSCEPTIBILITY

The high susceptibility of young animals to convulsions due to fevers--febrile seizures--has been ascribed by an NINDB grantee to an immaturity of the balance of water and electrolytes

(solutions which conduct electrical impulses) in the brain. Studies in animals show that susceptibility to febrile seizures can be increased or decreased by experimentally altering the brain's water and electrolyte balance. Observations of conditions which influence seizure susceptibility in children suggest that the findings may be clinically valid.

Investigations of the relationship between susceptibility to febrile seizures and age were conducted by Dr. J. Gordon Millichap, NINDB grantee and special trainee at the Albert Einstein College of Medicine, N.Y., and are reported in Neurology. Initial studies by Dr. Millichap demonstrated that the height of the temperature at which seizures occur may be used as an accurate index of seizure susceptibility.

To identify the mechanisms which may influence seizure threshold, hyperthermia was induced in groups of newborn, 1-month-old, and adult rats, and convulsive temperatures for each group were determined. Water and electrolytes in plasma and brain tissue were then examined in rats of the same age and strain, and results were correlated with seizure susceptibility in the corresponding groups. In the young animals, a significant correlation was found between high susceptibility to seizures and high intracellular water content of the brain. These susceptible animals were also found to have high levels of extracellular potassium and low levels of extracellular sodium.

To confirm these findings, the water and electrolyte balance in adult rats was experimentally altered to approximate that of the young rats, by such procedures as overhydration and glucose administration. When this was done, significant increases in seizure susceptibility occurred.

Clinical studies of children subject to febrile seizures suggest that similar changes in the water and electrolyte balance may be present. In addition, states of overhydration or salt depletion have been found to lower seizure thresholds in both children and adults.

CHEMICAL PATTERNS IN THE BRAIN USED TO CLASSIFY DRUG-INDUCED SEIZURES

Studies by NINDB grantees have demonstrated that certain types of cerebral seizures may be distinguished on the basis of specific chemical patterns in the brain, even though the electrographic patterns are alike. Although some chemical changes in the brain occur with all intense convulsions, other changes were found to be specific for the type of convulsive agent used. A tentative classification based on chemical patterns has been outlined for drug-induced seizures.

New data on seizures induced by four convulsive agents have been reported in Neurology by Drs. William E. Stone, Jean K. Tews, and Ellis N. Mitchell, University of Wisconsin Medical School. Changes in brain chemistry occurring during seizures were determined in dog cerebral tissue frozen

in situ. Analysis of some 15 cerebral chemicals led the investigators to classify these seizures into the following types.

The first chemically distinct type were seizures induced by anti-cholinesterase drugs. These seizures are recognized by the accumulation of acetylcholine in the brain. In addition, atropine was found to block this type, but not to affect any of the other types studied.

Seizures induced by fluoro-fatty acids, specifically fluoroacetate and fluorobutyrate, were also accompanied by specific changes in brain chemistry. Here, inhibition of the enzyme aconitase resulted in an accumulation of citrate in the brain, and large amounts of ammonia were also present.

A third possible category are seizures caused by picrotoxin and pentylenetetrazol. Although chemical changes were similar for both of these drugs, no major effects were found which accounted for their convulsive action. Convulsions due to pyridoxine deficiency represent another distinct type. Deficiencies of pyridoxine probably cause seizures by inhibiting the formation of gamma-aminobutyric acid in the brain.

Although many other known convulsants, such as strychnine and methionine sulfoximine, may cause specific chemical changes, additional studies with these compounds are necessary. The determination of chemical changes associated with induced seizure activity in animals may be of importance in defining some causative factors and possible therapies in human epilepsy.

HEADACHE

HUMORAL AGENT IMPLICATED IN VASCULAR HEADACHE OF MIGRAINE TYPE

An extremely powerful vasodilator, labeled "neurokinin," has been found in the body fluids of patients during severe and long-lasting vascular headache of the migraine type. In addition to the neurokinin, the body fluids contained an enzyme capable of forming neurokinin, termed neurokinin-forming enzyme (NFE). The increased content of these substances found locally could account for the pain associated with headaches of this type.

This clinical study, conducted by Drs. L. F. Chapman, A. O. Ramos, G. Silverman, H. G. Wolff, and Helen Goodell, B.S., at the New York Hospital-Cornell Medical Center, is reported in AMA Archives of Neurology.

During the painful phase of these attacks, the authors report, there is dilatation of the large and small blood vessels of the head both intra and extracranially, often more on one side than the other. In most attacks, however, the pain stems chiefly from the large sub-surface cranial arteries and their branches and aching pain is experienced when these vessels are distended, pulled upon, or displaced.

Intradermally injected tissue fluids containing mixtures of neurokinin and NFE induce pain, lower pain threshold, increase capillary permeability, and heighten vulnerability to injury. Also, the release or activation of neurokinin-forming enzyme and subsequent formation of neurokinin has been observed during neuronal excitation in man in a variety of circumstances. The potent hypotensive and vasodilator action of this substance and its formation during neuronal excitation suggest strongly that it serves in local vasomotor control for the nervous system and is also linked to migraine headache attacks.

CEREBROVASCULAR DISEASES

FACTORS UNDERLYING RECURRENT CEREBROVASCULAR ATTACKS DESCRIBED

Many recurring cerebrovascular attacks may be attributed to the failure of anastomotic small blood vessels attempting to compensate for stricture or occlusion of a major cerebral artery. Their failure may be caused by a variety of events including lowered systolic blood pressure, local hypertension, anomalous vascular distribution, and inadequacy of other major cerebral vessels.

A report by Dr. D. Denny-Brown of the Harvard Medical School in the A.M.A. Archives of Neurology suggests that analysis and treatment of factors which cause cerebrovascular episodes should precede anticoagulant or surgical therapy, and further definition of these causes may result in beneficial therapeutic measures.

Initial studies by Dr. Denny-Brown showed that recurring, temporary episodes of partial paralysis, formerly attributed to vascular spasms, were caused by a phenomenon which he called "carotid or basilar insufficiency." This syndrome occurs when a stricture or occlusion impedes the blood flow of one or more major arteries. Arterial function is then taken over by collateral, or

secondary arteries. Impairment of the collateral circulation at this time, however, was found to result in a "hemodynamic crisis," usually temporary and reversible, but which caused anatomical damage when severe. Hemodynamic crises occurred most frequently in patients with low systolic blood pressure, high blood viscosity, and anemia.

Subsequent studies were designed to investigate physiological factors which might cause the collateral circulation to fail and thus precipitate hemodynamic crises. In these experiements, the oxygen content of tissues in the monkey brain was measured by polarograph electrodes or stereoscopic microscope. Findings showed the occlusion of carotid or cerebral arteries caused an immediate drop in blood supplies to the area, with a resulting decrease in oxygen levels. Although the oxygen loss in normal animals was very great at first, it tended to rise slowly, reach a high or moderate recovery level.

Following carotid occlusion, when the monkey's blood pressure was lowered by withdrawal of blood, the small collateral vessels at first dilated, but quickly became constricted. The slow flow of blood resulted in the appearance of a phenomenon called "venous microstasis," characterized by clumping of red cells and stoppage of blood flow (stasis) in the very small venules. Although venous microstasis could be reversed by an increase of blood pressure, it resulted, if untreated, in microscopic intracortical hemorrhages, with a related death of affected nerve tissue.

The occurrence of venous stasis in the human brain is also related to low systolic blood pressure. In man, however, vascular occlusion may be incomplete, although the collateral insufficiency produced may result in considerable disturbance of function. Once a severe episode has occurred, attacks may cease, due to the decreased oxygen requirement in the affected tissues.

In managing cases of cerebrovascular insufficiency, therefore, it is essential to insure adequate blood pressure levels and oxygenation of arterial blood to prevent failure of the collateral circulation. Although anticoagulant therapy may be useful in preventing the initial clumping of red cells, this treatment has little subsequent effect.

Arterial spasms observed in hypertensive monkeys, while not related to insufficiency, were found to prevent adjustment in the collateral circulation. Unsuspected damage to the vertebral arteries in the neck has also been demonstrated to cause compensatory failure.

Recurrent, temporary cerebrovascular episodes may occur, however, in the absence of an insufficiency syndrome. Such cases, Dr. Denny-Brown suggests, may be caused by soft, friable emboli, probably composed of platelets and fibrin, which temporarily constrict blood vessels. Such platelet clumps, which appear to result from previous damage to a blood vessel, have been observed in experimental animals, and should be suspected when slight, frequent attacks occur. Although these clots have a natural tendency to fragment and disappear, anticoagulant therapy may in these cases have a beneficial effect.

Treatment for cerebrovascular insufficiency, Dr. Denny-Brown suggests, should be designed to counteract the immediate cause of symptoms. In the majority of cases he has observed, causative factors have been defined, and correction has been satisfactory in 50 percent without anticoagulant therapy or surgery. Although such therapy is of value in some cases, a thorough analysis of possible causative factors should be made before undertaking treatment.

NEW SYNDROME OF CIRCULATORY INSUFFICIENCY IN SPINAL CORD DESCRIBED

Temporary deficiencies of blood supply in the spinal cord, in patients with damaged blood vessels of the cord, have been studied and recognized as a syndrome similar to circulatory insufficiency of the brain. Although less common, the syndrome has been described in patients with spinal artery occlusion who exhibit episodic fluctuation of neurological symptoms. In addition, the investigators found that symptoms in these cases were temporarily aggravated by smoking.

The first definitive studies of transient vascular insufficiency of the spinal cord were conducted by Dr. C. Van Buskirk and M. Davidson, NINDB grantees at the University of Maryland School of Medicine, Baltimore, and were reported in the Southern Medical Journal.

Symptoms of occlusion of the anterior spinal artery, which usually occur abruptly, include partial paralysis, sensory loss, and possible muscular atrophy. However, patients usually show some degree of recovery, indicating that collateral, or secondary, circulation gradually substitutes for the damaged artery. In three cases studied, impaired collateral circulation in the damaged portion of the cord resulted in a temporary insufficiency syndrome, characterized by transient fluctuation of symptoms.

Since symptoms in two patients were temporarily aggravated by smoking, animal experiments were initiated to study the effect

of nicotine on the spinal circulation. Intravascular injection of nicotine in dogs was found to effect a significant, although temporary, reduction in spinal cord circulation. Thus, the authors conclude, the temporary neurological symptoms which occurred in patients after smoking resulted from the reduction by nicotine of the already impaired circulation.

In treating patients with spinal artery insufficiency, it is necessary to promote the collateral circulation and prevent its failure. Previous studies have suggested that the same factors influence spinal cord blood flow as influence cerebral circulation. Therefore, treatment should be aimed at insuring adequate cardiac function, avoidance of drops in blood pressure, intensive physical therapy, and a consideration of anticoagulant therapy. The investigators suggest that anticoagulant therapy be initiated only in carefully selected patients with a firmly established diagnosis.

NORMAL OCCURRENCE OF CONNECTIVE TISSUE IN CNS DESCRIBED

Strands of connective tissue fibers attached to adjacent blood vessels of the brain and spinal cord have been found in normal animals of 12 species, although many investigators have associated the fibers only with pathologic conditions. The recognition of the normal and widespread distribution of intervascular fibers will prevent erroneous diagnoses of such disorders as capillary fibrosis (where vessels are occluded by hyperplastic connective tissue fibers). In cerebral edema, however, stretching of these fibers may be a cause of impeded blood flow in small vessels.

Stained sections of brain and spinal cord tissue from normal animals were compared microscopically by Dr. J. Cammermeyer, Laboratory of Neuroanatomical Sciences, NINDB. Findings were reported in the Journal of Comparative Neurology. Intervascular fibers were found to vary in size, number, and distribution in different species and locations. Strands were innumerable in the pigeon; they were numerous and prominent in the dog, raccoon, monkey and cat; and less numerous in the rabbit, guinea pig, chinchilla, rat, hamster, squirrel, and mouse. No strands were found in the opossum, which has no capillary network.

Evidence that these fibers are derived from blood vessels is based on their staining qualities, intimate connections with vessels, and arrangement. Modification of the blood flow with normal maturation may result in regression and atrophy of scattered blood vessels, leaving connective tissue fibers bare.

The function of these fibers is not yet clear, except that they act as a support for the brain's vascular system. However, they should be considered in such pathological conditions as edema, where stretched fibers may constrict blood vessels. The high prevalence of intervascular strands in the spinal cord may also influence studies of the etiology of diseases of the cord.

EFFECTS OF OCCLUSION OF SMALL CEREBRAL ARTERIES DEMONSTRATED EXPERIMENTALLY

Studies of "traveling blood clots" in small arteries on the surface of the brain show that tissue damage occurs only if a branch of the artery which penetrates the cortex is occluded. If the "clot" does not lodge on a penetrating artery, complete occlusion of the surface (leptomeningeal) artery can occur without tissue disintegration (infarction) or breakdown of the natural barrier between blood and brain.

In conducting the experiments, NINDB grantees at the Bowman Gray School of Medicine, Winston-Salem, North Carolina, injected a steel sphere into a large artery of the dog brain, and localized it in a surface artery. Thus, the effects of a single, uniform "point" occlusion, where other vessels are unaltered, could be determined.

Drs. J. Kiffin Penry and Martin G. Netsky found that blockage of a penetrating artery characteristically caused two major, well-defined infarcts: one in the gray matter of the cortex, the other deep in the central white matter. The location and appearance of these infarcted areas was similar in each brain examined.

This phenomenon, they suggest, may be due to the structure of the brain's collateral, or secondary, circulation. In the penetrating arteries, the small capillary connections are not sufficient to compensate for the occluded vessel. Tissue damage in the gray matter may extend only to a depth where larger collateral vessels are located. But a sparseness of capillaries in the white matter again results in an area of damage.

The investigators noted that the infarct in the cortex was of a red, or hemorrhagic type, implying that a damaged blood-brain barrier allows a leakage of capillary blood cells. The deeper infarct was white, or anemic, possibly because of the poorer capillary supply. In previous studies of humans, a combination of red and white infarcts was attributed to the movement of a clot through the artery. But the steel sphere, which could be localized with certainty, was always located above both areas of damage.

Complete blockage of vessels without infarction has been described in other organs (such as the heart) in cases where the occlusion developed slowly. Yet occlusion in the dogs was sudden, necessitating an immediate development of collateral circulation. Although the collateral circulation of the leptomeningeal arteries of dogs has been shown to be similar to that of man, the authors indicate that occlusion of the same vessels in a human brain with atherosclerosis or arteriosclerosis might produce severe and irremediable damage. These findings were reported in the Archives of Neurology.

DISORDERS OF VISION

SIDE EFFECTS OF ENZYME USED IN CATARACT SURGERY EVALUATED

Alpha chymotrypsin, an enzyme which facilitates cataract surgery by fragmentation of the fibrils in the suspensory ligament of the lens (zonula fibers), is found to injure the endothelial lining of rabbit corneas, although the damage is usually temporary. This injury does not seem to be of a specific nature. Related studies show, for the first time, that endothelial cells multiply by mitosis, or indirect division.

The report of an NINDB committee investigating the use of alpha chymotrypsin was presented by Dr. Ludwig von Sallmann, Chief, Ophthalmology Branch, NINDB, at the annual meeting of the American Academy of Ophthalmology and Otolaryngology. The report summarized the findings of a cooperative study suggested by the Academy to investigate the effects of the enzyme on other parts of the eye.

In studies by Dr. von Sallmann, alpha chymotrypsin was infused into the anterior chamber of the eye of young rabbits, and removed after three minutes with washings of 0.9 percent saline solution. Subsequent examinations showed that the corneal endothelium was frequently injured. In some of the experiments, varying degrees of corneal clouding were found to occur. In these instances, large defects of the endothelium could be detected microscopically. Damage confined to a small area usually disappeared after 24 hours, although extensive corneal clouding sometimes persisted for more than five weeks. Control animals infused solely with 0.9 sodium chloride also occasionally exhibited endothelial damage.

Because of the difficulty of examining the endothelium, a new technique was devised in which the entire cell population of this tissue could be studied. Flat mounts of the whole endothelium were prepared by dissecting layers of the excised corneas under a stereomicroscope. A significant result of this technique was the demonstration that cells in the endothelium of adult animals multiply by mitosis, contrary to previous assumptions that division was amitotic, or by direct cleavage. In animals with extensive injuries, cell regeneration by mitotic activity was greatly increased.

Results of preliminary tests of the allergic effects of alpha chymotrypsin, conducted by Dr. G. van Alphen of the Ophthalmology Branch, NINDB, were included in the report. Here, guinea pigs were sensitized by repeated injections of the enzyme. Challenge doses a month after sensitization did not produce noticeable allergic responses. These studies may indicate that adverse reactions to alpha chymotrypsin are unlikely to occur in patients previously treated with the enzyme for other disorders, or where it is used a second time for a cataract operation.

Also described were electromicroscopic studies of the effect of alpha chymotrypsin on the lens conducted by NINDB grantees A. P. Ley, A. S. Holmberg, and T. Yamashita of the Washington University School of Medicine, and reported in the American Journal of Ophthalmology. The investigators found that the zonules, supporting structures of the lens, are fragmented into uniform segments three to five minutes after alpha chymotrypsin is administered. These experiments, conducted with monkey and human eyes, contradict previous findings that the enzyme merely weakens the fibers of the zonules.

VENOUS CIRCULATION OF INTACT LIVING EYE VIEWED BY NEW TECHNIQUE

The application of an intricate radiographical technique to intact living eyes has enabled an NINDB investigator to describe the complex intraocular venous circulation of the cat eye. Little known anatomical details and theories of circulation, which may be applicable to human eyes, have been clarified by the use of this technique.

Although radiographic methods have been used to study the vascular system of the living brain, the valuable tool previously had not been applied successfully to the vessels within the eye. Known as intraocular venography, the new technique is of potential value in diagnosing some eye disorders and in studying such conditions as congestive glaucoma, in which vascular disturbances occur. The study, by Dr. Bruce E. Cohan of the NINDB Ophthalmology Branch, was reported in A.M.A. Archives of Ophthalmology.

In performing the experiments, the anterior ciliary vein of the cat was exposed, divided, and cannulated with a needle attached to tubing

and a syringe. The vein was then perfused with a radiopaque material and film packets were placed flat in a space provided by the removal of the cat's nasal orbital wall. During perfusion, exposures were made by appropriate positioning of a central X-ray beam.

An interpretation of these radiographs enabled Dr. Cohan to design a schematic composite of the circulation pattern and to describe little known details of the intraocular venous system. Since other comparative studies have shown that the intraocular circulation of the cat and dog resembles that of man, Dr. Cohan suggests that the findings of this study may be valid for the human eye.

Radiographic evidence failed to confirm the presence of previously described swellings and constrictions in the veins of the choroid, a vascular layer between the sclera and the retina. It is possible, however, that the elevated venous pressure during the experimental procedure obscures such caliber changes which might be present under physiologic conditions. In addition, high-speed perfusion resulted in the appearance of radiopaque material in the anterior aqueous chamber--between the cornea and the iris--implying a connection between the chamber and the intrascleral veins. The intrascleral veins were also found to communicate with the veins of the ciliary body, which connects with the iris.

The study showed that when perfusion rates were increased, intraocular pressure also increased substantially. In several experiments air was not completely flushed from the tubing and harmless amounts were introduced into the circulation. The finding that large amounts of air were trapped in the vortex sinus when intraocular pressure was elevated suggested that outflow from the vortex vein had been impeded. According to Dr. Cohan, this tends to confirm the many speculations that venous outflow is strangled under conditions of elevated intraocular pressure.

NEW CONCEPTS OF DRUG ACTION IN LOWERING INTRAOCULAR PRESSURE REPORTED

A surprising correlation between venous and intraocular pressure in the eye has been described as a result of a new technique for experimentally measuring these pressures without altering the relationship between them. Studies have shown that Diamox (acetazolamide), a drug used to lower intraocular pressure in glaucoma, produces parallel decreases in both intraocular and venous pressure in the cat eye. Diamox was also found to act selectively, without interfering with the general blood pressure.

A description of the experimental technique and subsequent studies of the effects of Diamox on the eye were presented by Dr. Frank J. Macri, Ophthalmology Branch, NINDB, at the meeting of the American Society for Pharmacology and Experimental Therapeutics.

Preliminary to the drug study, casts of the aqueous outflow veins of the cat eye were made to demonstrate connections with the systemic veins. The effect of Diamox was then measured by simultaneously recording the aqueous pressure in the anterior chamber and the venous pressure in the anterior ciliary or one of the vortex veins. Continuous measurements were made before, during and after the administration of the drug.

In previous studies, eye and venous pressures had not been determined simultaneously. Thus the venous pressure was essentially determined on an occluded vein while the eye pressure was obtained under conditions of an unimpaired circulation. A technique was, therefore, devised where either the anterior ciliary or the vortex pressure was measured simultaneously with the intraocular pressure and the blood pressure. The comparative experiments showed that no changes in the eye's "responsiveness" resulted from the cannulation of veins for purposes of measurement.

When Diamox was administered intravenously, expected decreases in eye pressure occurred. In addition, simultaneous decreases in eye venous pressure were recorded, which were parallel to intraocular changes in time of onset and in magnitude. In addition to performing the experiments in living animals, studies were made on isolated arterially perfused eyes. Similar correlations of intraocular and venous pressure were found under this condition and Diamox was still effective in lowering these pressures. Since under these conditions decreases may be ascribed to the alkalinity of the drug used, a comparable buffered solution was administered by continuous perfusion to the isolated eyes. Lack of pressure decreases under these conditions confirmed that Diamox acts directly on the eye.

Intraocular pressures in one eye of each of 39 cats were then plotted against corresponding venous values before and after Diamox administration. Decreases after the use of Diamox were statistically significant for all cases. A significant linear correlation between venous and eye pressures was found, a relationship which was unchanged by the use of the drug. According to Dr. Macri, this finding could indicate that the vasculature plays a role of greater significance in the maintenance of ocular pressure than has been attributed to it in the past.

Although no conclusions are warranted relating to the mechanism by which Diamox causes the venous pressure to fall, two possibilities are indicated. Venous pressure may decrease as a result of lowering the eye pressure, since Diamox is known to decrease aqueous inflow. Or, intraocular decreases may be due to falls of venous pressure, implying that some mechanism produces a lessened blood volume. Further studies on these intricate phenomena may contribute to the understanding of intraocular pressure regulation.

IMPROVED METHOD FOR DIAGNOSING MALIGNANT TUMORS WITHIN THE EYE REPORTED

Isotope tracer methods for detecting malignant ocular tumors have been implemented by NINDB investigators who have devised criteria for successfully applying these methods to tumors in the posterior half of the eye. Tracer methods that are used to locate tumor sites in the front of the eye have previously been ineffective in locating posterior tumors. The improved method for localizing tumors in the posterior choroid has been reported in the A.M.A. Archives of Ophthalmology by Dr. J. O'Rourke, formerly of the NINDB Ophthalmology Branch, now at Georgetown University, and Eleanor Collins, NINDB.

Eight patients, each with evidence of a tumor beneath the retina in the posterior half of the eye, were studied. Radiophosphate was administered intravenously to the patients 24 hours before uptake measurements were made. Beta-emissions were counted for one minute in each of four posterior and anterior quadrants of the eye. After the tumors were excised, pathologic sections were examined to determine the cell type and volume of each tumor.

Results showed that the routine anterior counting procedure localized the posterior malignancy correctly in only one of eight cases, and the average radioactivity of the tumor was only 25.3 percent above the mean of individual quadrant counts. In six cases where the new criteria were used for posterior countings, the tumor was located correctly in all instances. The average increase in tumor radioactivity was 74 percent above the mean. The investigators also found that tumors larger than 300 cu. mm. and those with epithelioid cells (which are associated with high malignancy) retained greater amounts of radioactivity. However, factors such as density of overlying tissue made it difficult to equate tumor size with radioactivity emitted.

As a result of these findings, the authors propose that different methods be used to detect posterior malignancies than are now used for locating ocular tumors. They suggest that one minute counts of quadrants in both the front and back hemispheres of the eye be made, and that posterior counts be taken from bare sclera, following surgical exposure and muscle detachment. A positive diagnosis of a posterior tumor can be made if the total radioactivity in the back hemisphere exceeds the total anterior values by more than 40 percent. Radioactivity detected over the tumor site should also exceed the mean of posterior counts by more than 40 percent. In addition, the point of highest radioactivity should correspond with the site of retinal detachment.

This method of detecting posterior malignancies is highly accurate since more radioactivity can be counted, thereby reducing the chances of error. In addition, the actual area which the tumor has invaded can be more accurately determined. The technique will also be of value in advancing operating time, since the difficulties previously encountered in detecting posterior malignancies often delayed surgery.

NINDB STUDIES CLARIFY ROLE OF NERVE PATHWAYS IN REGULATING EYE PRESSURE

Sensory neurons in the eye which respond to increased intraocular pressure are not involved directly in the maintenance of normal eye pressure in the cat. In addition, NINDB studies have indicated that, over short periods of time, intraocular pressure is not regulated by the central nervous system. Although the existence of a neural center which regulates eye pressure has been suggested to explain clinical observations, a reflex pathway has never been demonstrated.

To determine if such pathways exist, investigators recorded the afferent impulses--from the eye to the brain--from the ciliary nerves of the cat, which carry the sensory nerve fibers of the eye as well as its autonomic supply. Experiments were conducted on isolated, perfused cat eyes, and findings were confirmed in intact, anesthetized animals.

In both types of experiments, no spontaneous neural activity was observed which could be related to normal intraocular pressure levels. Increases in pressure, however, resulted in increased activity of the larger sensory fibers of the long ciliary nerves. The impulses evoked by raising the pressure, suggest the authors, may be due to the stretching of nerve fibers in the outer coats of the eyeball.

Indirect evidence for central regulation of intraocular pressure also was sought in intact animals. Stimulation of the third and fifth cranial nerves (which give rise to the ciliary nerves) was found to produce transient changes in eye pressure. However, when the normal pressure was increased by infusion of fluid into the eye's anterior chamber, no changes in the efferent activity--from the brain to the eye--were observed. In addition, blocking the nerves by local anesthesia did not affect the normal pressure or the response to small imposed changes of pressure.

In view of these findings and of the small amount of activity evoked by pressure changes, the authors conclude that short term regulation of intraocular pressure in the cat is probably not a function of the central nervous system. However, the study does not exclude the role of neural activity in regulating normal eye

pressure, since the techniques used did not permit the study of the activity of small fibers or of long-term changes in pressure. The study was conducted by Dr. P. P. Lele, formerly of the NINDB Ophthalmology Branch, and now at Massachusetts General Hospital, and Patricia Grimes, Ophthalmology Branch, and is reported in Experimental Neurology.

VISUAL ADAPTATION TO THE DARK DEPENDS ON RECOVERY OF PIGMENT

A study which shows how human eyes adjust to the dark after exposure to light, conducted by an NINDB ophthalmologist, has proven that dark adaptation is directly related to the regeneration of a photosensitive pigment in the retina known as rhodopsin or "visual purple."

Further conclusions substantiate findings from animal studies indicating a linear relationship between recovery of night vision and regeneration of rhodopsin, regardless of differences in light exposure (bleaching) or subsequent time in the dark.

An ingenious method for measuring both dark adaptation threshold (point of minimum vision) and rhodopsin levels in the same eye has been developed by Dr. W. A. H. Rushton, former Visiting Scientist, Ophthalmology Branch, NINDB, who reported the results of this work at the meeting of the Association for Research in Ophthalmology. By this method, predetermined fractions of rhodopsin in specified retinal regions can be bleached with lights of varying intensities, and the extent of bleaching and rhodopsin recovery can be measured by retinal densitometry, which determines pigment density.

In typical experiments, different intensities of rhodopsin bleaching, ranging from total to 25 percent, were produced and studied. The subject alternated between the densitometer and an apparatus for measuring dark adaption, which employed yellow and green filters to indicate the point of transition from cones (day vision) to rods (twilight vision). In all cases, the point of transition and the return of minimum vision occurred when rhodopsin was about 90 percent regenerated.

Dr. Rushton concludes that the dark adaptation threshold is probably dependent on the fraction of "opsin," a chemical component of rhodopsin, which has not at a given time combined with "retinin," a yellow pigment, to form rhodopsin. This theory is supported by electroretinographic studies in animals which indicate that the amount of rhodopsin remaining after bleaching or vitamin A deficiency has a linear relation to the level of the visual threshold.

VERTIGO

DESTRUCTIVE THERAPY EVALUATED FOR RELIEF OF INCAPACITATING SYMPTOMS OF MENIERE'S DISEASE

Incapacitating symptoms of Meniere's disease affecting one ear can be relieved by a simple and effective surgical technique known as the transtympanic labyrinthotomy. This technique, which destroys the function of the inner ear, is recommended only when medical therapy fails to prevent recurring and severe attacks of the disorder.

The criteria for undertaking destructive therapy and for determining the appropriate surgical or drug technique are examined by Dr. Harold F. Schuknecht, NINDB grantee, Associate Surgeon, Division of Otolaryngology, Henry Ford Hospital, Detroit, in an article published in the A.M.A. Archives of Otolaryngology.

Dr. Schuknecht cautions that destructive therapy should be considered only when Meniere's disease is conclusively diagnosed, when the vertigo and nausea are incapacitating, and when medical regimens fail to provide symptomatic relief.

When destruction is indicated, and hearing in the healthy ear is good, unilateral involvement may be treated effectively by surgical transtympanic labyrinthotomy, or, as Dr. Schuknecht suggests, by intratympanic streptomycin injection. Since these procedures result in hearing loss in the diseased ear, existence of a progressive disease in the other ear must be ruled out.

Destructive therapy by either technique is not recommended unless the threshold loss for speech reception is at least 40 decibels and the speech discrimination score is not better than 40 percent.

According to Dr. Schuknecht, the surgical method is simpler and quicker. The intratympanic streptomycin treatment, which the investigator first tried on a series of cats before its use on humans, causes patient discomfort and requires daily injections for a week. Its only real advantage is that it requires no general anesthetic. Destruction of vestibular function by either method must be complete, otherwise the disease will persist in the residual sense organs.

Where the disease affects both ears -- only in about 10 percent of the cases -- the technique employed should assure preservation of the existing hearing. Because of its remarkable selectivity, parenterally administered streptomycin sulfate assures the safest destruction of the labyrinth without impairing auditory sense.

Dr. Schuknecht stresses that pre-treatment audiograms and quantitative caloric tests should be given at frequent intervals beginning ten days before starting out-patient treatment, with particular emphasis upon caloric results. Injections to a point just short of total destruction of the vestibular system have been found by Dr. Schuknecht to produce optimum relief while avoiding the prolonged ataxia. To assure careful observation, the patient should be hospitalized after a week of out-patient treatment. It will be helpful to the patient to understand the nature of the temporary post-treatment disability--principally ataxia--which will gradually improve.

Dr. Schuknecht concludes that carefully administered parenteral streptomycin therapy can effectively relieve incapacitating symptoms of bilateral Meniere's disease. In cases of unilateral involvement, however, an equally effective result can be achieved by surgical ablation with less subsequent vestibular upset.

PROGRAM HIGHLIGHTS

NEW DEVELOPMENTS IN NINDB'S COLLABORATIVE PERINATAL PROJECT

For the past four and a half years, including two and a half years of intensive preparation and pretesting, NINDB has directed a long-term collaborative research attack on cerebral palsy, mental retardation, and other neurological and sensory disorders of infancy and childhood. The project is the first large-scale effort to collect information on the many factors which may cause neurological and sensory disorders. It is also the first large research program to collect detailed information during pregnancy, labor, delivery, and the early life of the child rather than after disorders occur.

The project will eventually involve some 50,000 pregnant women and their children in all parts of the country. Working on the project at 15 private hospitals and universities are obstetricians, pediatricians, neurologists, and specialists in at least a dozen other medical and related scientific disciplines. Their common objective is to determine whether there is a relationship between the events and influences present during pregnancy, labor, delivery, and the first month of life, and the later appearance of neurological or sensory disorders in some cases.

Since the main phase of the project was started in January 1959, there have been three major developments. First, a strong and closely knit organization of the 15 collaborating institutions and the central office has been forged. Second, the procedures for collecting data on the pregnant women and their children have been improved. And third, the number of women and children enrolled in the project has steadily increased: by the end of October 1960, study records had been received on about 12,274 mothers and 6,725 children.

PERINATAL RESEARCH EXHIBITS DISPLAYED

To describe NINDB's Collaborative Project, two new scientific program exhibits, entitled "The Fateful Months When Life Begins" and "40,000 Mothers and Their Babies," were prepared this year and shown at the annual meetings of fifteen professional societies.

The exhibits provide brief statements of the relationship between conditions affecting parents and the occurrence of abnormalities in their offspring, as well as some of the etiological factors which may result in deviations in pregnancy outcome.

PUERTO RICO PRIMATE STUDIES ENTER FOURTH YEAR

Major scientific contributions relating to the developing nervous system and oxygen deficiencies at birth have resulted from primate studies conducted by NINDB's Laboratory of Perinatal Physiology, Puerto Rico. Now in its fourth year of operation, the project has achieved its initial objective by establishing conclusively that monkeys asphyxiated at birth develop symptoms which resemble mental retardation and cerebral palsy. Current research is aimed at developing means of prolonging survival of asphyxiated monkeys and preventing resulting brain damage.

The studies are designed to complement the aims of the Institute's Collaborative Project on perinatal pathology in humans. At present, scientists are producing asphyxia in monkeys under more standard conditions, and investigating the numerous physiological changes which result. New observations have been made of brain pathways over which nerve impulses travel. Behavioral studies have shown marked differences between animals born and reared in captivity and those in the free-ranging colony.

Related accomplishments include the standardization of neurological examinations for newborn monkeys, establishment of normal growth and development patterns, descriptions of structural changes in the brains of asphyxiated monkeys, and important observation of behavior and learning activities.

The Puerto Rico facility has been made available to several other scientific groups in this country and abroad, including other NIH Institutes, for collaborative or individual studies. Outstanding studies conducted by Dr. Geoffrey Dawes and others, Nuffield Institute for Medical Research, Oxford, England, have obtained for the first time accurate records of the oxygen supply of the fetus, which has wide implications for causes of fetal suffocation in the human. Plans are being made to allow other interested investigators to use these animals and facilities for primate research, and to develop the laboratory as a center for experimental studies of numerous adverse conditions which may cause neurological disorders.

MATERNAL AND CHILD CARE MISSION TO THE USSR

The second NINDB-sponsored mission of six American scientists to the USSR has surveyed Soviet progress in the field of maternal and child care.

The 30-day tour was part of a scientific-cultural exchange program between the U.S. and the Soviet Union. A group of Russian

specialists will make a similar study of maternal and child care in this country at a later date.

Members of the U.S. delegation were Drs. Stewart H. Clifford, Boston Lying-In Hospital; Allan C. Barnes, Johns Hopkins Medical School; Katherine Bain, Children's Bureau, DHEW; B. G. Greenberg, University of North Carolina; Edith Potter, Chicago Lying-In Hospital; and Fred S. Rosen, Children's Hospital, Boston.

A report which details the findings of the first NINDB-sponsored medical exchange mission to the Soviet Union has recently been published. It contains observations on Soviet research activities in the physiology and pharmacology of the nervous system by six scientists who toured Russian research institutions in 1958. Copies of the extensive report (PHS publication No. 800) are available on request from the NINDB Information Office.

ADVISORY GROUP FORMED TO STUDY NEEDS IN CEREBROVASCULAR RESEARCH

To aid in outlining and implementing a national research program in cerebrovascular diseases, NINDB early in the year appointed seven neurological scientists to serve as a Cerebrovascular Program Advisory Group.

Before recruiting manpower and stimulating research on a national scale, the group has delineated the role of the neurological scientist in the study of vascular diseases of the brain. They have indicated that a major problem in the vascular field is the interpretation and measurement of the harmful effect of vascular disease on the organ concerned. They recommended that the "primary role in devising, implementing, and evaluating all forms of research in cerebrovascular disease must reside in the collective disciplines known as neurological science." A resolution to this effect was adopted by the National Advisory Neurological Diseases and Blindness Council as part of its policy in cerebrovascular research.

PROGRESS REPORTED IN ANEURYSM STUDY

An accelerated pace has been reported in NINDB's Cooperative Study of Intracranial Aneurysms and Subarachnoid Hemorrhages, begun early in 1958 and scheduled to be completed in 1962. The study, designed to give certain basic information and to point out lines for future research activities, is acquiring a large reservoir of clinical data.

Although specifically directed toward the analysis of subarachnoid hemorrhage and aneurysm, this accumulation of data is

pertinent to and potentially available for the study of many facets of the general problem of cerebrovascular disease.

By the middle of 1960, the study had compiled a total of 1,000 reports of subarachnoid hemorrhage, 450 of which have been proven to be caused by intracranial aneurysms.

When a sufficient number of cases have been reported, the project will determine which clinical types of patients suffering from aneurysms are best suited for particular medical and surgical therapies, and favorable sites for surgical removal of aneurysms.

VARYING OPINIONS RESULT FROM ANTICOAGULANT STUDY

Results of NINDB's Cooperative Anticoagulant Project, which began late in 1957, indicate that the use of anticoagulant drugs is certainly feasible, but may involve definite risks and problems. The aim of the project is to evaluate the usefulness of anticoagulants in preventing "little strokes," in warding off the occurrence or recurrence of major attacks, and in reducing the neurological deficit which may result. A study at one cooperating institution concluded that anticoagulant therapy did not substantially affect mortality from strokes, whereas another study has indicated that anticoagulant therapy is beneficial in treating certain types of cerebrovascular disease.

A future study will compare the effects of anticoagulants alone with anticoagulants plus fibrinolytic agents. Previous studies compared anticoagulants with controls.

INTERNATIONAL CONFERENCE ON VASCULAR DISEASE OF THE BRAIN

Research progress and future needs relating to vascular disorders of the brain, including findings from NINDB cooperative studies, were outlined by international authorities who attended a two-day conference in Miami. The conference, jointly sponsored by the NINDB Advisory Council and the American Academy of Neurology, followed the annual meeting of the AAN, and was attended by over 500 scientists. Reports were presented by investigators from the U.S., England, Canada, France, Belgium, Denmark, and Sweden.

The proceedings of the conference will be examined in detail by a selected group of authorities to determine the areas which would benefit from initiated or expanded research.

PLANNING OF INTERNATIONAL CEREBROVASCULAR STUDY SUPPORTED BY GRANT

A research grant for the planning of an international study of the epidemiology and neuropathology of strokes was awarded by NINDB to the World Federation of Neurology with headquarters in Antwerp, Belgium. The Federation, an international professional organization, will enlist the cooperation of its 48 member societies in conducting the study.

The project proposes to investigate factors which cause strokes in different national, ethnic, racial, and cultural groups throughout the world. During the planning phase, uniform reporting methods will be developed to evaluate lesions in the vessels of stroke patients. Means of selecting uniform patient samples must also be determined, so that results of studies in different countries can be meaningfully compared.

At a conference in Antwerp, study participants and the Advisory Group agreed upon tentative operating protocols that have been developed to standardize study results. They recommended that a central laboratory be established for uniform interpretation of specimens from cooperating institutions. To initiate the pilot study, the group suggested that methods be devised to begin collecting specimens at research centers in Oslo, Norway, and Turin, Italy. Arrangements will be made to evaluate institutions in other countries for inclusion in the study.

FIRST INTERNATIONAL SYMPOSIUM ON PARKINSON'S DISEASE CO-SPONSORED BY NINDB

Fifty-one of the world's foremost authorities on Parkinson's disease, convening at Arden House, concluded that a central registry of Parkinson victims would facilitate systematic study and analysis of the ailment. Highlighting the 3-day symposium, the first international event of its kind, were the findings of five specialized panels, presented at an open meeting.

New estimates of the prevalence of Parkinson's disease were presented, with the estimate that one out of forty Americans, or 2.4 percent of the population, will develop the disorder. At present, some 34,000 new cases are reported in this country annually.

The current deficit in knowledge of Parkinson's disease was attributed in part to the lack of standardized approaches and of uniform terminology. In addition, too many Parkinson cases are treated without careful study or are not reported. Participants agreed, however, that an immediate step toward

eventual eradication would be the establishment of a complete registry of case histories, to be available to scientists everywhere, for study and analysis in the search for possible causes, prevention, and cures.

Differing opinions relating to the merits of surgical therapy were presented, as well as basic knowledge of nerve connections within the brain. In addition, the neurologists agreed that most drugs now in use for relieving Parkinson symptoms are generally unsatisfactory. Summary opinions were also developed by the symposium concerning possible hereditary factors in Parkinson's disease, the location of brain impairment, or malfunction, and the experimental production of the disease in animals.

DRUG THERAPY IN NEUROLOGICAL AND SENSORY DISORDERS EVALUATED AT NATIONAL SYMPOSIUM

Higher and more uniform standards for clinically testing drugs for neurological and sensory disorders were discussed at a symposium, sponsored by the NINDB Advisory Council, at the University of Wisconsin. One hundred outstanding neurologists met with eight of the Nation's leading biostatisticians to set up standards for clinical trials of drugs now in use, as well as for future therapy.

Panel groups in eight specific disorders reviewed the broad aspects of therapy and proposed methods for most effective evaluation of drugs in each disease area. Sessions were held to discuss epilepsy, glaucoma, multiple sclerosis, vertigo, Parkinsonism, headaches, muscle diseases, and neuropathies and neuralgias.

Preceding the panel meetings, objectives of the symposium were outlined and the importance of human clinical trials in screening proposed drugs was emphasized. The clinical neurologist was advised to cooperate with experts in designing and evaluating experimental trials. The general session also included talks by representatives of the pharmaceutical industry and the Food and Drug Administration who defined problems in testing and evolving new drugs.

The symposium was highlighted by reports summarizing the findings of the eight specialized groups. Specific recommendations relating to the design of clinical drug trials in each area were presented. In general, the participants concluded that additional work in this essential area was imperative. They also agreed that methodology studies should be undertaken to develop accurate and

consistent definitions and categorizations of disease entities to be studied, to validate available methods of examination of patients and to establish uniform criteria of drug effectiveness.

COOPERATIVE STUDY ON GLAUCOMA DETECTION CONTINUES

An NINDB-supported cooperative study to evaluate methods for screening and diagnosing glaucoma has entered its second year at four research centers throughout the nation. Techniques currently applied to the detection and identification of glaucoma are being evaluated at the Wilmer Institute, Johns Hopkins University Hospital, Baltimore; the Moffitt Eye Hospital, University of California Medical School, San Francisco; the Department of Ophthalmology, State University of Iowa, Iowa City; and the Department of Ophthalmology, Washington University School of Medicine, St. Louis, Missouri.

A report on a Glaucoma Family Study, undertaken by NINDB grantees at the latter university, appears in the October 1960 issue of the American Journal of Ophthalmology. In a twelve month period, 110 siblings, children, and parents of known glaucoma patients have been studied.

GLAUCOMA STUDIES PRESENTED IN AWARD-WINNING EXHIBIT

Relationships between the severity of eye chamber angle changes in glaucoma and of certain systemic disorders are illustrated by an NINDB grantee exhibit which was awarded the Hektoen Gold Medal at the annual meeting of the American Medical Association in Miami Beach. The Medal is awarded on the basis of originality and excellence of presentation for an exhibit of original investigation.

The exhibit is entitled "Chamber Angle Anomalies in Developmental Glaucoma and in Systemic Mesodermal Disorders." It presents findings indicating that certain connective tissue disorders, such as Marfan's syndrome and idiopathic scoliosis, are consistently accompanied by abnormalities in the connective tissue of the eye's chamber angle. These abnormalities are structurally similar to chamber angle anomalies in the two basic types of developmental glaucoma, suggesting that the conditions may have a common cause.

Also illustrated are microscopic and histochemical studies which indicate that both the systemic disorders and chamber angle anomalies may result from inborn metabolic errors that cause weakening of the ground substance supporting connective tissue cells and fibers.

The Hektoen Gold Medal was presented to Drs. Hermann M. Burian, Gunter K. von Noordon, Ignacio V. Ponseti, and Mr. Edwin Lee Allen, of the State University of Iowa College of Medicine, Iowa City.

PILOT STUDY OF UVEITIS ORGANIZED

In a new approach to the problem of uveitis, leading ophthalmologists attending an NINDB-supported conference on this disorder organized and initiated a pilot study for analyzing records of uveitis patients. The initial study, which will include some 500 case histories, will be used in organizing a larger project to be conducted at four major eye clinics. Analysis of numerous case histories may provide a basis for determining possible causes, means of prevention, and cures for the blinding disease.

Conference participants also evaluated a proposed classification system for uveitis and retinitis. To determine its practical value, they suggested that the system be used for a year by clinicians working in these disease areas. Dr. Alson E. Braley of the State University of Iowa, a member of the NINDB Advisory Council, was chairman of the conference.

EFFECTS OF RADIATION ON THE NERVOUS SYSTEM EVALUATED AT INTERNATIONAL SYMPOSIUM

The response of the nervous system to ionizing radiation was discussed by 42 authorities from seven countries (including Russia) at a symposium at Northwestern University, jointly supported by NINDB and the Atomic Energy Commission.

Five half-day sessions, each followed by a discussion period, covered the effects of radiation on the developing nervous system, particle irradiation (particularly the use of high energy rays in neurosurgery), and histopathological, functional, and psychological changes resulting from radiation exposure. Dr. Ray Snider, an NINDB grantee at Northwestern, was chairman of the symposium, which was attended by some 340 scientists.

Differing opinions relating to the effects of low dosage radiation were presented. Studies indicating that a very low radiation level produced congenital abnormalities in embryo rats were reported by Dr. Roberts Rugh of Columbia University. Low dosage radiation was also reported to increase the incidence of audiogenic seizures in affected mice. Another study, reported by Dr. Orville T. Bailey, University of Illinois, a member of the NINDB Neurology Postgraduate Training Committee, suggested that

observed effects of radiation on the nervous system are primary, rather than secondary, to vascular changes. This observation confirms the study of Dr. Milton Brightman, Laboratory of Neuroanatomical Sciences, NINDB.

A paper presented by Dr. Marcel Monnier of Basel, Switzerland, revealed that low doses of radiation altered electrical discharge patterns in the brains of mice. Other reports concerned the necessity for exact definition of radiation sensitivity, the relation of age and species to radiation damage, and the effects of radiation on nerve impulse transmission. Participants also reported that, in general, motivation and motor activity are decreased by radiation exposure.

RESEARCH OPPORTUNITIES IN CHRONIC DISEASE HOSPITALS

The research potential in chronic disease hospitals throughout the nation was considered at a two-day NINDB conference attended by thirty directors of research, superintendents of hospitals, and chiefs of neurology.

The agenda included a review of the present patient load, types of patients, and disorders involved. Participants were urged to outline types of research opportunities within their institutions and to suggest ideas for their fullest possible exploitation.

Consideration was given to unique conditions existing in certain chronic disease hospitals for the study of controlled populations over extended periods of time. These facilities provide an ideal environment for the close study of the life history of chronic disease, the accurate evaluation of response to therapy, and the precise clinical, pathological or chemical classification of disease.

The handicaps of research programs in the chronic disease hospitals include the serious limitation of personnel with time available for research and geographic and professional isolation. A possible solution might be found in the pooling of resources -- the creation of a few specialized laboratories to which selected patients, or material from rare cases might be sent for study.

TRAINING GRANTS IN NEUROSURGERY INAUGURATED BY NINDB

Departments of Neurosurgery now are eligible for training grants under NINDB s Grants and Awards Programs. The postdoctoral grants in neurosurgery, initiated this year, are awarded to qualified neurosurgical centers. These programs are designed to provide research training for individuals in preparation for

careers in teaching and research. They will provide support for neurosurgical training on the same basis and under the same policies adopted for all other neurological and sensory disciplines and specialties.

NEW TEACHER-INVESTIGATOR TRAINING GRANT PROGRAM ESTABLISHED

Institutions that do not have full-scale training programs can take advantage of a new NINDB program of teacher-investigator training grants for all areas of research related to neurology.

The first award under the program was made to Dr. Philip P. Ellis, University of Colorado, for work in opthalmology. Other applications are under consideration in areas ranging from neurophysiology to neurosurgery.

These awards are intended to add a fulltime staff member to institutions who will serve as a focus for neurological research and teaching in his department. Titles under which these persons will work will be left to the discretion of the individual institutions.

PROCEEDINGS OF CONFERENCE ON HEARING PUBLISHED

A published monograph entitled "Neural Mechanisms of the Auditory and Vestibular Systems," has resulted from an earlier NINDB-sponsored conference at Bethesda.

Among the specific topics discussed in detail are electron micrographic studies, histopathologic studies and role of the cochlea, anatomy and physiology of the auditory cortex, studies with implanted electrodes, and afferent and efferent auditory connections.

The book is edited by Drs. Grant L. Rasmussen and William F. Windle, NINDB staff members, and is published by Charles C Thomas.

NEW BROCHURES ON CEREBRAL PALSY AND MONGOLISM PUBLISHED

A new booklet titled "Cerebral Palsy--Hope through Research," published this year, explains in laymen's terms the nature of cerebral palsy and related problems, and suggests where help may be sought. Research at NINDB and other institutions is described as the basis of hope for discovery of preventives and improved treatments. This leaflet is listed as PHS publication No. 713, Health Information Series No. 95.

"Mongolism--Hope through Research" is another brochure for laymen printed this year. Research in all problems of mental retardation at NINDB and other research centers is described. Because of demands from States with Spanish-speaking populations, the booklet was translated and published in Spanish. The leaflet, which has a selected list of references, is listed as PHS publication No. 720, Health Information Series No. 94.

Both brochures are available on request from NINDB or from the Government Printing Office.

Items of Interest on Program Developments and Research

Studies Conducted and Supported by the Clinical Center

PHYSIOLOGICAL MONITORING SYSTEM FOR CRITICALLY ILL PATIENTS

In keeping with its mission of originating new hospital methods, the staff of the Clinical Center has developed an electronic device for monitoring the physiological functions of post-operative and acutely ill patients. Help with technical details and production of the finished product was obtained from Gulton Industries, Inc., Metuchen, N. J. It was financed by the National Cancer Institute.

Development of new medical and surgical techniques, plus common use of ever more potent drugs has greatly increased the nursing time required for such patients. It is no longer unusual to find a patient requiring two special nurses to supply the intensive care needed post-operatively. The time of one of these nurses is taken up almost exclusively in frequent recording of vital signs.

Space medicine techniques for measuring certain of the vital functions have been adapted to clinical use. It was decided that the five signs most needed in following an acutely ill patient's condition are: (1) blood pressure; (2) pulse; (3) temperature; (4) respiration; (5) arterial oxygen saturation.

To be of value, the monitoring system had to have an alarm which would indicate which function in which patient had exceeded the tolerable range set for that patient. It had to have some type of visual record to indicate the values received into the equipment from the patient. It had to be simple in its operation so that it would not require a highly skilled technician in its use. It had to have a "fail safe" system which would indicate when the unit itself was not functioning properly. Lastly, it had to be small enough to fit into the patient's room. It was also determined that to be practical the system should monitor

four patients, the number of beds available in the largest recovery room in the Clinical Center. The device was installed late in the month of November.

A great value of the physiological monitor is that it records all measurements taken. A "print out" is part of the equipment and it gives a permanent written record in alpha-numerical form for each patient at whatever interval is determined to be necessary. The form may be pasted directly into the nurse's notes on the patient's chart and thus saves the time necessary for transcribing.

It is stressed that the Clinical Center does not feel this system will, in any way, reduce nursing hours necessary for the care of patients. It is an auxiliary aid for the nurse which allows her to perform the functions that only a nurse can perform, but it should make for better and more efficient patient care.

RADIOPHARMACEUTICAL SERVICE

Until this year radioactive drug products used at the Clinical Center were prepared by individual investigators or were received from manufacturers. Sometimes the manufactured product was not entirely suitable to the particular need of the investigator. To keep in step with the ever-growing demand for this type of pharmaceutical, and to give investigators more time to devote to their primary function, a Radiopharmaceutical Service has been established.

For the most part, investigational type radioactive drug forms are prepared by this Service for diagnostic use, but it also compounds some few oral drugs for treatment in dosage forms. Preparation of these products is governed by the same controls and accuracy as all other drugs available in the Pharmacy Department of the Clinical Center. They are given safety tests for pyrogenicity, sterility, and radioactive assay. The latter test is performed by the Radiation Safety Office.

In cooperation with clinicians and laboratory investigators, radioactive drug products which are not available from any other source are developed and synthesized. Examples are the development of chromium 51 labeled human serum albumin; I 131 labeled gamma globulin; oral fat emulsion with 131; and improvement of previously developed polyvinyl pyrrolidone - I 131 injection, and krypton 85 injection. The Radiopharmaceutical Service cooperates with industry in working toward a safer, purer product for patients. This is a mutually beneficial arrangement in which information is freely passed back and forth since all knowledge gained is to mutual advantage and serves the general cause of research.

AUTOMATION IN CLINICAL PATHOLOGY DEPARTMENT

Laboratory consultation and services for patient care are provided to the Clinical Center by its Clinical Pathology Department in the fields of clinical chemistry, hematology, microbiology and special diagnostic procedures. Because of the research mission of the Clinical Center, laboratory tests for patients participating in research projects are far greater in number and variation, with more exacting quality control, than those required for care of patients in general hospitals.

The Department has continued to improve the efficiency of current tests and to introduce new procedures which will be helpful to the clinical staff in patient care and clinical research. For example, the use of automatic instrumentation in this Department has proceeded at a rapid pace.

The Hematology Service has developed an automatic attachment for the modified Coulter Electronic Cell Counter which was installed last year. This attachment enables the technician to obtain size distribution curves of red cells in seconds, and provides an accurate automatic technique for counting platelets. Although experimentation with the device is still in progress, it is being used now on a limited number of studies such as blood analyses for certain leukemia projects.

In the Clinical Chemistry Section, measurements of blood glucose and blood urea nitrogen, serum phosphatase, and serum bicarbonate are now being determined routinely by the Autoanalyzer. Many of the other routine chemical estimations are performed partially on an automatic basis. Examples of these tests are measurements of phosphatase, creatinine, total protein, and albumin. All of these procedures employ a systematic method for the measurement of samples and reagents and are read on a semi-automatic recording photometer.

Active programs are in progress to automate the flame photometer of serum cations, including sodium phosphatase and calcium.

HIGHLIGHTS OF PROGRESS

IN RESEARCH ON

BIOLOGICS

1960

Items of Interest on Program Developments and Research Studies

Conducted by the Division of Biologics Standards

The Division of Biologics Standards administers those provisions of the Public Health Service Act pertaining to the control of biological products in interstate and foreign commerce. Two types of licenses--establishment and product--are issued for the production and sale of such products, following a determination by the Division that the prescribed standards for safety, purity, and potency have been met. In the first eleven months of the calendar year, 188 establishment licenses and 278 product licenses were in effect, the latter covering 1228 specific products. Tests of approximately 8000 individual lots of various biological products were made, ranging from relatively simple sterility tests to potency determinations involving both animal and tissue culture tests.

Of equal importance in ensuring the continued safety, purity, and potency of these products is the preparation and distribution of physical reference standards to manufacturers and laboratories engaged in biological standardization throughout the world. Approximately 4,000 vials of such standard preparations are distributed each year by the Division.

The Division's research is a vital part of its program. Continuous efforts are made to improve and to develop new testing procedures, as well as to improve methods of preserving and storing physical reference standards, to correlate them with standards established in other countries, and to develop new ones.

RESEARCH STUDIES

CHARACTERIZATION OF ATTENUATED POLIOVIRUSES IN THE ORAL VACCINE AND AFTER PASSAGE IN MAN

The most recent findings in continuing studies of the properties of attenuated poliovirus strains, and the characteristics of these strains before and after gastrointestinal passage in man, were

reported by Drs. Ruth Kirschstein and Samuel Baron, Laboratory of Viral Immunology, at the Second International Conference on Live Poliovirus Vaccines.

Low neurovirulence for monkeys is considered a prerequisite for virus strains to be used in live poliovirus vaccines--the proposed strains were selected on this basis. A comparison of the neurovirulence of the attenuated poliovirus vaccine strains, when inoculated directly into the central nervous system, was reported last year.

Current studies show that some attenuated virus strains retain their ability to invade the central nervous system of monkeys when inoculated intramuscularly. This was demonstrated by histologic lesions in sections of the brain and spinal cord and by paralysis of some monkeys. Two routes of spread from the site of inoculation to the CNS appeared to be possible--either through the blood stream or along the peripheral nerves. It was concluded that the nerve supply of the inoculated muscle was the only way it could be prevented.

An additional requisite for virus strains to be used in live poliovirus vaccines is stability of low neurovirulence characteristics during vaccine production and human passage.

The results of these continuing studies indicate that 1) the temperature character as determined in a plaque system is very often correlated with monkey neurovirulence; 2) minor laboratory manipulation of the vaccine strains often results in marked alteration of the virulence characteristics; 3) virus obtained from stool examinations of persons fed vaccine virus exhibited a high frequency of increased monkey neurovirulence.

Results of these studies have been applied as additional criteria for selection of strains for oral vaccines.

POLIOVIRUS INHIBITOR FOUND IN RHESUS MONKEY CENTRAL NERVOUS SYSTEM

During studies in which rhesus monkeys were inoculated intracerebrally and intraspinally with attenuated poliovirus--an established neurovirulence testing procedure--an unexpectedly low rate of virus isolation from the central nervous system (CNS) of the infected monkeys was observed. Moreover, when infected CNS tissue was plaque-assayed on monkey kidney cell cultures, more virus plaques appeared at higher dilutions of CNS tissue than at lower dilutions, strongly indicationg virus inhibition.

In a study undertaken to determine the nature of this phenomenon,

Dr. Samuel Baron and co-workers, Laboratory of Viral Immunology, found that the inhibition was not directed against the virus but affected viral multiplication by altering the host cell (Science).

When suspensions of lumbar or cervical cord tissue were assayed for poliovirus content, virus was recovered from only 30 percent of the infected monkey, whereas in previous studies, recovery of virus from CNS tissue of infected monkeys was readily obtained. Further tests revealed that suspensions of CNS tissue could inhibit up to 900 plaque-forming units of poliovirus in cell culture. Moreover, the inhibitor was found in the CNS suspensions of normal as well as infected monkeys.

Although the inhibitory effect of monkey tissue was demonstrated against each of the three types of poliovirus, no significant inhibition of Coxsackie A9 and ECHO 12 viruses could be demonstrated. This indicates some degree of specificity of the inhibition. Furthermore, when the CNS tissue suspensions were combined with large amounts of poliovirus and then diluted for plaque assay, no inhibition was demonstrated, indicating that the inhibition did not irreversibly neutralize the virus, but in some way affected viral multiplication by acting on the host cells.

NEUROVIRULENCE OF TYPE 1 POLIOVIRUS CARRIED BY RIBONUCLEIC ACID (RNA)

Previous studies by several investigators have shown that the ribonucleic acid (RNA) of of a number of animal viruses, when extracted from the virus particle, will initiate infection in tissue cell cultures. Although these studies indicate that the infectivity is due to the RNA itself and not to residual virus particles, it was not known whether subtle differences in the genetic makeup of the virus could be transmitted by the RNA. One such difference would by the virulence of the virus particles for animals.

Drs. Paul Gerber and Ruth Kirschstein, Laboratory of Viral Immunology, have presented evidence that genetic information controlling neurovirulence and other characteristics as well as infectivity, are transmitted by the viral RNA (Journal of Experimental Medicine).

The study was made with two Type 1 poliovirus strains which have been adapted to mice by Dr. C. P. Li, Laboratory of Virology and Rickettsiology. The LSa strain is virulent for mice; the LSc strain is an avirulent variant developed from the LSa strain. Although the RNA from each strain had the same infectivity for cell cultures, the RNA from the virulent strain was highly neurotropic for mice when inoculated intraspinally, whereas the RNA from the attenuated strain was completely avirulent for mice.

Additional evidence that the inheritable character of virulence was maintained following passage of viral RNA was derived from an experiment with rabbit kidney cell cultures infected with LSc-RNA and LSa-RNA

Although intact poliovirus cannot infect rabbit kidney cells, it was possible to induce limited infection with the isolated RNA. The viral progeny from such infected cells, when inoculated intraspinally into mice, produced infections characteristic of the parent RNA virus.

Moreover, when the progeny of the Mahoney-RNA in monkey kidney cell cultures were incubated at 40° C, their reproductive capacity (t marker) remained undiminished. In contrast, the RNA extracted from the highly attenuated LSc virus failed to synthesize complete virus at that temperature.

CELL CULTURES FOUND TO DEVELOP RESISTANCE TO PHOTODYNAMIC ACTION

During studies of poliovirus by plaque assay technique, workers observed that the top layer of monkey kidney culture would frequently degenerate within 48 hours after application of overlay medium. Since this raised a problem in interpretation of viral assays, a study was undertaken to determine the cause of this degeneration.

Experiments were conducted to determine the variables of temperature, dye, and light. The results indicated that the combination of neutral red dye and light was the damaging factor. However, when cells stained with neutral red were incubated in the dark overnight, cell degeneration did not occur following exposure to light. Although other workers have described the occurrence of photodynamic action on cells, development of resistance to photodynamic action has not been reported previously.

From a practical point of view, the discovery makes possible the elimination of unexpected cell degeneration during virus assay by shielding the infected cell cultures from light during the initial 12-16 hours of the 6-day incubation period.

Of academic interest is the mechanism by which cell resistance developed. There were two possibilities: the resistance could be due to a change in the cells or to alteration of the dye. Cultures which had developed resistance to light after incubation with neutral red for 48 hours were also found to be resistant to sensitizing action of the addition of fresh dye. This indicated that loss of sensitization was probably due to a change in the cells rather than alteration of the absorbed neutral red. Furthermore, when neutral red was extracted from resistant cell cultures and applied to fresh cells, the cell cultures died after exposure to light, indicating that there was no alteration in the dye.

A complete report of this work, co-authored by Alice M. Gochenour

and Samuel Baron, Laboratory of Viral Immunology, appeared in the Proceedings of the Society for Experimental Biology and Medicine.

INFECTIVE NUCLEIC ACID (DNA) ISOLATED FROM POLYOMA VIRUS

The first evidence that an isolated nucleic acid will turn a normal cell into a cancerous cell has been reported by Sloan-Kettering Institute and NIH scientists.

Preliminary work on the isolation of infectious deoxyribonucleic acid from SE polyoma-infected tissue cultures was published early this year in the Proceedings of the National Academy of Sciences by Drs. G. A. Di Mayorca, Sloan-Kettering Institute; Bernice Eddy, DBS; Sarah Stewart, NCI; et al. (The SE polyoma virus, originally isolated from leukemic mice by Drs. Stewart and Eddy several years ago, has to date produced more than 20 types of tumors in a number of species of laboratory animals.)

Isolation of infectious nucleic acids, either from purified viruses or virus-infected animal or plant tissue, have been reported by various workers, with positive identification of RNA as the infectious agent, but no well-substantiated findings of DNA. The induction of tumors in mice with preparations of mixed nucleic acids from leukemic mice also has not been clarified (Hayes and Latarjet).

The most convincing evidence for the successful isolation of an infectious nucleic acid depends on its sensitivity to a specific nuclease. The authors report the isolation from mouse embryo cultures infected with SE polyoma virus of an infectious nucleic acid which is resistant to the action of ribonuclease (RNase) but which is destroyed by deoxyribonuclease (DNase). The intact polyoma virus is resistant to both enzymes.

The virus was grown in mouse embryo cells in tissue culture and recovered from the fluid medium of the tissue culture. Then the protein jacket of the virus was removed and the viral nucleic acid isolated and injected into embryonic cells in tissue culture. When the medium from this tissue culture was injected into hamsters, it caused tumors in 100 percent of the animals within 18 days. When the enzyme DNase was added to the isolated nucleic acid it became non-infective, but was resistant to the action of RNase. Results of direct inoculation of nucleic acid preparations into animals will be reported later.

The results of these studies suggest that the nucleic acid of the SE

polyoma virus is DNA, and that the latter is infective when extracted and purified from a virus suspension.

JUICE OF THE ABALONE FOUND TO POSSESS ANTIMICROBIAL EFFECT

The search for antiviral and antibacterial agents has been largely concentrated in the field of microbial products. However, the abundance and variety of organic materials in sea water, and the known ability of certain mollusks to filter and concentrate suspended solids of their aquatic milieu, led Dr. C. P. Li, Laboratory of Virology and Rickettsiology, to study the abalone as a potential source of antimicrobial agents.

As reported in the Proceedings of the Society for Experimental Biology and Medicine, Dr. Li has demonstrated the antimicrobial action of fresh abalone juice against Staphylococcus aureus, as well as a protective effect of canned abalone juice against experimental poliomyelitis in mice.

At the beginning of his study, Dr. Li tested the inhibiting effect of canned abalone juice on S. aureus, but found the results to be inconsistent. Fresh abalone juice, however, demonstrated a consistent and marked antimicrobial activity against S. aureus. Furthermore, when the juice was heated to 95°C for 45 minutes, or dialyzed against water overnight, no impairment of its effect was evidenced.

In testing the effects of abalone juice in experimental poliomyelitis, Dr. Li inoculated groups of Swiss mice with each of the three types of poliovirus, and fed the gastropod product to the treated mice in their regular diet. The rates of paralysis and death were 20 to 50 percent lower in the treated animals than in the controls. Moreover, the incubation periods were, in general, several days longer in the treated mice.

The thermostability and nondialyzability of this agent suggests that it is not an ordinary antibiotic. One of the chief advantages of its possible use for therapeutic purposes is that it is edible as a food. Further work on purification and concentration of the agent is in progress.

VIRUS GROWTH OF CELL CULTURES MAINTAINED WITH SKIM MILK MEDIUM

A study to determine the sensitivity of various tissue cells in culture maintained with skim milk medium indicates that the medium is free of inhibitor

for infectivity and permits full sensitivity to infection by viruses tested.

The development by Dr. Samuel Baron, Laboratory of Viral Immunology, (Science, 1958) of a serum-free medium containing skim milk which effectively maintains a wide variety of cell cultures, led to the present study of viral sensitivity of various cell cultures in this medium.

Comparative titrations by Dr. Baron and co-workers of 22 different virus strains showed that the infectivity titers of ECHO, Coxsackie, poliomyelitis, influenza, herpes, measles, and vaccinia viruses in the presence of skim milk medium were equal to or greater than those obtained when established maintenance medium was used.

Strains of adenovirus maintained in skim milk showed a lower infectivity for the cells than in established maintenance media. Experiments to determine whether the skim milk acts directly to repress adenovirus multiplication or whether it lacks the cell-mediated enhancing factor of other media indicate that its inhibitory action was probably an effect on the tissue culture cells rather than an antiviral effect. Similar cell-mediated effects have been observed by other investigators.

The overall results of this study confirm the suitability of skim milk as a maintenance medium. Full details of the study by Alice Gochenour and Dr. Samuel Baron, Laboratory of Viral Immunology, were reported in the Proceedings of the Society of Experimental Biology and Medicine.

SELECTIVE INACTIVATION OF ANIMAL VIRUSES IN SUSPENSIONS OF LIVE POLIOVIRUS

Dr. Caspar Hiatt and co-workers, Laboratory of Biophysics and Biochemistry, discovered that when minute quantities of toluidine blue dye (1 to 10 parts per million) were added to monkey kidney tissue culture fluids containing both poliovirus and B-virus, the latter became the target of subsequent bombardment with light energy and was destroyed, while poliovirus showed no significant loss of infectivity.

In 1958, Dr. Hiatt, in studying the effect of light on viruses in the present of toluidine blue, had found that while many animal viruses become sensitized for photodynami inactivation on subsequent exposure to visible light, others--including poliovirus--are highly resistant to this treatment.

B-virus is one of the 40-odd wild viruses that have been encountered

in the routine testing of the killed poliomyelitis vaccine. Since these contaminating agents are even more readily inactivated by formaldehyde than are the poliovirus, they introduce no hazard in the killed vaccine. However, elaborate precautions and tests have had to be devised to make certain that they are not present in live poliovirus vaccine since no way has been found to inactivate them without also inactivating the poliovirus.

Little is known of the pathogenicity for many of these animal viruses, except for the B-virus which, although it causes only a mild illness in monkeys, is capable of producing fatal encephalitis in human beings. Sixteen such infections, 14 of them fatal, have occurred since 1937 in laboratories in England, Canada, and the United States among personnel engaged in research with monkeys and monkey tissue material.

Since a number of animal viruses can now be inactivated by this selective method, it may be of practical use as an added safeguard in the manufacture of live poliovirus vaccine.

FORMATION OF TRUE ANTIBODY BY HYPOGAMMAGLOBULINEMIC PATIENTS INDICATED

Despite the characteristic limitation of gamma globulin formation, persons with hypogammaglobulinemia are usually resistant to a second attack of a viral infection. Since antibody formation in these patients is usually undetectable by standard tests, resistance generally has been attributed to a non-antibody immune mechanism.

In a collaborative study, DBS and NIAID scientists have detected what appears to be naturally acquired neutralizing antibody to polio, ECHO, and Coxsackie viruses in the serum of persons with hypogammaglobulinemia (reported in the New England Journal of Medicine).

Using a highly sensitive technique (immunoinactivation plaque-neutralization tests), the serums of two patients with congenital hypogammaglobulinemia and of five with acquired hypogammaglobulinemia were found to have neutralizing capacity for a number of enteroviruses. The plaque-neutralizing material in the serums of these patients has properties of true antibody: 1) it is heat stable, and 2) the reaction of the antibody with the virus is time-dependent--two factors which do not obtain with nonspecific inhibitors.

In a subsequent study, Dr. Baron and co-workers, Laboratory of Viral Immunology, tested the antibody response of four hypogammaglobulinemic patients to a 10-ml inoculation of poliomyelitis vaccine, followed by a 1-ml dose one month later. Three of the patients, prior to inoculation, had low antibody titers to all three types of polio virus. Following inoculation, no significant rise in antibody occurred in two but the third had

a small but definite increase to types 2 and 3 after the second inoculation. The fourth patient had pre-existing antibody to types 2 and 3 polio virus, but none to type 1. Following the first inoculation, extremely small amounts of type 1 antibody, which diminished during the third week and increased significantly following the second inoculation, were detectable in this patient. No change in concentration of type 3 antibody was observed.

The result of this second study indicates that hypogammaglobulinemic patients may have a primary or secondary antibody response following exposure to an antigen, but the maximum obtainable antibody levels are severely limited. One possible interpretation of these findings is that the low levels of antibody in these patients are sufficient to confer immunity to viral diseases but insufficient to confer immunity to many bacterial and fungal diseases.

DETERIORATION OF STORED ALBUMIN DETECTED BY SENSITIVE METHOD

The problem of stability of normal human serum albumin during storage is of practical as well as theoretical interest. For example, when held in a stockpile, it must be able to withstand storage and shipment under various temperatures without significant change.

A long-term project has been undertaken in the Laboratory of Blood and Blood Products to determine what changes occur in stored albumin, the conditions affecting these changes, and the most suitable methods for detecting protein alteration. One phase of this study, reported by Dr. J. S. Finlayson and co-workers, in the Journal of Clinical Investigation, shows that the co-ordinated use of chromatography and ultracentrifugation provides a sensitive method for detecting alteration in normal human serum albumin during long-term storage.

The study was carried out on commercial human albumin preparations which had been stored for at least 5 years under various conditions. Indications were that the extent of changes occurring in albumin during storage are dependent on both the nature of the material used for preparation and the conditions of storage.

There was minimal difference between the chromatographic behavior of the albumin samples prepared from citrated whole plasma and stored for 5 years at 5° C and the chromatographic behavior of fresh albumin samples either prepared commercially or in DBS laboratories. However, albumin stored for ten years at 5° C or for five years at 32° C (with or without prior storage) demonstrated marked differences in chromatographic properties, as did albumin prepared from ultraviolet-irradiated dried plasma.

Fractions of albumin separated by chromatography were subjected

to ultracentrifugation. The concomitant use of both techniques permitted measurement of protein components not readily observed by using either technique alone.

The clinical significance of any of the differences noted is not presently known; however, the alterations in physical-chemical character of albumin under certain conditions obviously warrant further investigation of the problem.

Studies initiated by Dr. J. T. Tripp, Chief of the Laboratory of Blood and Blood Products, using other techniques such as electrophoresis and viscosity are being continued. The work of Dr. Finlayson and co-workers indicates, however, that the chromatography-ultracentrifugation technique offers a sensitive method for the detection of protein changes in stored serum albumin.

PROGRESS IN DEVELOPMENT OF MEASLES VACCINE

In order to assess and correlate the progress made in the development of a measles vaccine since their last meeting in 1958, investigators from university and pharmaceutical laboratories were invited to present their findings at a day-long conference at the Division of Biologics Standards on November 18. The meeting, chaired by Dr. Roderick Murray, Director, DBS, was attended by more than 50 virologists.

Dr. John Enders, whose work with measles virus established the principles for an attenuated measles vaccine, summarized his recent experience with the Edmonston strain of virus grown in chick embryo cells. Clinical data presented by other groups using live vaccine made from this strain gave evidence of its capacity to elicit satisfactory protection against natural measles as well as specific antibodies among the vaccines.

The Enders vaccine was shown by three groups of investigators to give 100% protection in children exposed to natural virulent measles virus, while the unvaccinated controls showed the expected high incidence of the disease. Evidence of satisfactory antibody titer at the end of 18 months was also presented. To date, close to 1000 children have been given this vaccine.

The Enders vaccine, in most cases, caused some clinical reaction--principally fever. However, when administered concurrently with gamma globulin, such reaction was virtually nonexistent. This so-called active-passive immunization was favored by two groups of investigators but additional studies are necessary.

Although various methods of preparing the vaccine have been used, propagation in chick embryo tissue culture is preferred, since adventitious agents which might be pathogenic for man are less likely to be encountered.

There has been some question in the past whether a live vaccine

of sufficiently high titer could be produced which would maintain its potency during storage. It is now generally agreed that it is possible to stabilize the vaccine in the dry state.

Studies with both experimental animals and human subjects have given assurance that the Enders strain is devoid of deleterious neurotropic effect. Dr. Frederic A. Gibbs, University of Illinois School of Medicine, and his colleagues, using electroencephalography for determining neurologic reaction showed that approximately 50% of children develop significant abnormalities in their EEG patterns during the course of natural measles. In contrast, no such changes were evident in children inoculated with the Enders attenuated vaccine. This would indicate that central nervous system involvement, which does occur in natural measles, rarely if ever occurs with attenuated vaccine.

Dr. Harry Meyer, Laboratory of Virology and Rickettsiology, DBS, outlined the work being done in DBS laboratories in developing serologic tests for detection of measles antibodies. Methods for preparation of a dried reference serum are also being investigated for use in both the complement fixation and neutralization tests. The standardization of gamma globulin preparations for measles antibody content is also planned.

Work will be continued by the various investigative groups in acquiring a larger clinical experience. DBS will continue to coordinate these studies and to encourage the acquisition of new data.

DBS RESEARCH STUDY PRESENTED AT SEATO-NIH CHOLERA CONFERENCE

A mouse protection test for the assay of cholera vaccine, developed by Dr. John C. Feeley and Dr. Margaret Pittman, Laboratory of Bacterial Products, was presented at the first of the conferences on cholera, sponsored by the South East Asia Treaty Organization. The 4-day conference was held in December in Dacca at the recently established laboratory for cholera research in East Pakistan, where the disease is endemic.

International reference cholera vaccines of the Inaba and Ogawa types of Cholera comma were established in 1948 by the World Health Organization but without a unit of potency, because of failure of collaborative assays. Two years ago, WHO's interest was renewed and a Study Group in which Dr. Pittman participated formulated recommended requirements for cholera vaccine. The need for comparing laboratory potency values with prophylactic efficacy in man was emphasized and the development of a mouse protective test to evaluate the potency of cholera vaccine quantitatively was initiated by DBS in anticipation of clinical trials to be

carried out by the WHO.

The major emphasis in Dr. Feeley's work has been the standardization of the variables which influence the test, as well as the selection and preservation of a U. S. reference vaccine to determine its relative potency. Difficulty has been encountered in preserving the potency of the reference vaccine during freeze-drying and various methods are now under investigation.

Using the mouse protection test, reproducible relative potencies of vaccines prepared in other countries, as well as the WHO reference preparations, have been determined. It has also been possible to determine the mouse cross-protection of the two C. comma types, Ogawa and Inaba--a problem which has received much attention in the past.

Although the DBS test is feasible for the evaluation of the mouse protective potency of cholera vaccine, clinical trials of vaccine of known laboratory potency must be carried out before its relation to epidemiologic effectiveness can be evaluated.

PROGRAM DEVELOPMENTS

DBS BUILDING DEDICATED JUNE THIRTIETH

The formal dedication of the Division of Biologics Standards building took place on June 30. His Majesty Bhumibol Adulyadej, King of Thailand, officially dedicated the building by unveiling an inscribed bronze plaque, which will be placed in the main entrance of the building.

Their Majesties, King Bhumibol and Queen Sirikit, who arrived for a four-day state visit in Washington on June 28, were guests at a luncheon given by Secretary and Mrs. Flemming at Stone House preceding the dedication ceremonies.

The invitation to participate in the dedication of the new DBS laboratories was extended to King Bhumibol because of his active role in health measures in his own country and his interest in the South East Asia Treaty Organization--National Institutes of Health Cholera Research Project.

During the 1958-1959 cholera epidemic in Thailand, the work of U. S. and Thai scientists demonstrated, through greatly reduced death rate, the value of collaborative research. The U. S. Cholera Research Advisory group which visited the SEATO and other Asian countries last year was granted an audience by King Bhumibol in Bangkok. The group was headed by Dr. Joseph E. Smadel, former

NIH Associate Director for Intramural Research, who assumed his new duties as Chief of the DBS Laboratory of Virology and Rickettsiology in July.

STERILITY TEST PROCEDURES FOR BIOLOGICAL PRODUCTS REVISED

The revision of one of the basic tests designed to ensure the purity of all licensed biological products reflects recent advances in manufacturing procedures as well as in scientific achievement.

The revised procedures for sterility testing, which manufacturers of biological products subject to control under the Public Health Service Act are required to follow, became effective on April 15. Redesignated as Section 73.73 of the Public Health Service Regulations, these requirements are designed to ensure the freedom from extraneous microorganisms of all licensed biological products. The revision represents a composite scientific opinion based on years of research.

The timeliness of the adoption of United States sterility test standards is emphasized by the recent recommendations of the World Health Organization. The participation of Dr. Margaret Pittman, Chief, Laboratory of Bacterial Products, DBS, in the WHO Study Group which formulated these recommendations at the Geneva Conference in 1958, facilitated the efforts of DBS in drafting comparable requirements. Although the U. S. standards are more detailed and specific, they are consistent with those recommended by WHO. This should be of considerable help to those U. S. producers of biologics engaged in foreign commerce, as well as to foreign manufacturers who export biologics to this country, since the latter must also be licensed to comply with U. S. standards.

BRITISH LABORATORIES STUDY DBS LIVE POLIOVIRUS RESEARCH

Indicating the world-wide interest in the control problems peculiar to the live poliovirus vaccines, three British virologists visited DBS laboratories early in January. Representing their respective laboratories in the field of live poliovirus vaccine research, were Dr. Alan P. Goffe, of the Burroughs-Wellcome Laboratories, Kent; Dr. J. A. Dudgeon, of the Virus Research Unit, Glaxo Laboratories, London; and Dr. Thomas Beswick, of the Medical Research Council Laboratories, London.

Of general interest were slides showing the histologic lesions

of the brains and spinal cords of monkeys inoculated with live attenuated poliovirus. The slides corroborated the data reported by DBS scientists at the World Health Organization-Pan American Health Organization Live Poliovirus Conference last June. The report, "Comparative Virulence for Rhesus Monkeys of Poliovirus Strains Used for Oral Administration," by Dr. Murray, et al, was published in the Proceedings of the First International Conference on Live Poliovirus Vaccines.

SOVIET-AMERICAN POLIO CONFERENCE MUTUALLY PROFITABLE

The first mission to take place under the U. S.-U. S. S. R. Exchange Agreement of November 1959 was the Soviet-American meeting on poliomyelitis control held in Moscow early in May. Attended by 13 American and 15 Russian scientists, the meeting was devoted chiefly to technical discussions of research, production, and testing of live poliovirus vaccines.

The Russians reported the successful use of the oral vaccine developed by Dr. Albert Sabin, Director of Research at the Children's Hospital, Cincinnati. The Russian-produced Sabin vaccine had been given to 60 million Russians two months to 20 years of age. Russian scientists voiced the hope that this method of control will completely eradicate the disease in Russia within the next year or two. They agreed, however, that further studies of effectiveness and duration of effectiveness were needed.

The American scientists described U. S. experience with the killed poliomyelitis vaccine, which had been given to more than 90 million persons in this country, and the field trials with the live poliovirus vaccines of Drs. Sabin, Koprowski, and Cox.

The discussions indicated that, while the proposed production and testing standards of both countries are parallel in most respects, certain differences exist. It was agreed that further exchange of information and comparative studies is desirable and that such information be provided to the World Health Organization with a view to the ultimate development of international recommendations.

Americans attending the meeting were Dr. David Price, Chief of the U. S. delegation and personal representative of the Surgeon General; Dr. Sabin; Dr. Koprowski; Dr. Benyesh-Melnick and Dr. Joseph E. Melnick, Baylor University School of Medicine; Dr. Theodore E. Boyd, National Foundation; Dr. Victor Cabasso, Lederle Laboratories; Dr. Eugene Flipse, University of Miami School of Medicine; Dr. John P. Fox, Institute for Public Health Research,

New York City; Dr. Thomas Francis, University of Michigan; Dr. Herman Kleinman, Minnesota Health Department; Dr. Alexander Langmuir, CDC; and Dr. Roderick Murray, Director, DBS.

The Russian delegation was headed by Dr. V. M. Zhdanov, Secretary of the Academy of Medical Sciences of the U. S. S. R., and included Dr. M. P. Chumakov, Director of the Institute for Poliomyelitis Research in Moscow, and other virologists, epidemiologists, and research scientists.

RECOMMENDATIONS FOR LIVE POLIOVIRUS VACCINE PRODUCTION

After extensive study of all three candidate strains (Sabin, Cox, and Koprowski) the PHS Committee on Live Poliovirus Vaccines met August 19 with technical representatives of interested drug firms and with DBS staff to review the proposed requirements for the manufacture and testing of live poliovirus vaccine.

The Committee recommended the Sabin Type I and II strains as possessing the most favorable laboratory and field characteristics. The Sabin Type III was recommended as being satisfactory from the standpoint of neurovirulence, although it has less than optimal immunogenic capacity and shows a tendency to change its neurovirulence characteristics after passage in man. Neurovirulence for monkeys is considered the most important laboratory criterion available, and the intrathalamic test in monkeys has been recommended for adoption as a test for neurovirulence.

The Committee also recommended that when it becomes available, live poliovirus vaccine be used on a community rather than on an individual basis. This recommendation was made principally because of the potentiality of reversion to virulence of the live virus strains. In the field studies, the vaccine has been largely employed in mass administrations with the vaccine given simultaneously to the population thus permitting little opportunity for serial human transmission of the virus; or the vaccine has been administered during a time of the year when "wild" virus strains have usually shown limited capacity for spread.

The Surgeon General announced his acceptance of the Committee's recommendations in a press conference on August 24. Problems raised by the Committee regarding the optimal use of the vaccine are to be taken up with appropriate advisory groups, such as the State and Territorial Health officers and representatives of the medical and health professions and of the voluntary health agencies.

The recommendations, which were made available to those

manufacturers interested in the eventual production of the vaccine, will serve as the basis for the eventual adoption of regulations for live poliovirus vaccine. Manufacturers who make application, demonstrating the necessary experience and ability to produce a vaccine which conforms with the regulations, will be licensed.

It is not anticipated that the live poliovirus vaccine will be available in substantial supply before mid-1961.

LIVE POLIOVIRUS VACCINE RECOMMENDATIONS WELL RECEIVED ABROAD

Recommendations of PHS Committee on Live Poliovirus Vaccine were presented by Dr. Roderick Murray, Director, DBS, at the Sixth International Congress on Microbiological Standardization in Weisbaden in September. Members of the Congress appeared well satisfied with the progress made in the United States in the development of standards for live poliovirus vaccine. Dr. Murray discussed the recent recommendations of the PHS Committee on Live Poliovirus Vaccine as they affect the production of the vaccine. Copies of the recommendations were distributed to members of the Congress.

Dr. Albert B. Sabin, of the University of Cincinnati, also spoke on the subject of live poliovirus vaccine. Dr. Sabin summarized his work and described the technique he uses for intraspinal inoculation of monkeys.

Scientists from more than 20 nations attended the Congress, which serves as a means for exchange of scientific information. Although essentially composed of European biological manufacturers and interested representatives of control agencies and microbiological laboratories, the presentations at the annual meeting cover a rather broad area of the biological field, including human and veterinary biological products as well as such problems as the testing of disinfectants.

The two Russian scientists invited to present papers were unable to attend the conference.

EXCHANGE OF SCIENTIFIC INFORMATION ENHANCES DBS RESEARCH PROGRAM

The exchange of ideas and technical knowledge with other laboratories working in related areas has proved to be of inestimable value to the DBS research programs.

Dr. Gerald L. Van Hoosier, Laboratory of Virology and Immunology, returned to DBS in August after a year's study under Dr. Edwin H. Lennette, chief of the Viral and Rickettsial Disease Laboratory

HIGHLIGHTS OF PROGRESS

OF RESEARCH IN

GENERAL MEDICAL AND BIOLOGICAL SCIENCES

1960

Items of Interest on Research Studies Supported by the

Division of General Medical Sciences

The Division of General Medical Sciences was established at the National Institutes of Health in 1958 as an agency for the administration of grants programs. A prime objective of these programs is to strengthen research which will foster systematic knowledge of the natural phenomena which underlie organic disease generally. With this, the strengthening of clinical research and academic and technological resources oriented more directly to the conquest of disease is an increasingly important function of the Division's grants programs.

Unlike the Institutes of NIH which are oriented to specific diseases, the responsibility of the DGMS encompasses inquiry into the structures, processes and relationships which comprise the entire organism and constitute the organic and physical substrate of all our major disease problems. Appropriate inquiry into any aspect of the natural order which shows promise of yielding new knowledge of the organic factors which underlie disease may warrant DGMS support. This may include the design of analytical instruments, tools and methods, and the development of new perceptual constructs and systems of inductive reasoning which show promise of engendering such knowledge by their proposed application.

In addition, the Division administers NIH grants programs for training investigators in the disciplines basic to medical research, and it directs the NIH Center for Aging Research. The Division also has responsibility for certain disease-oriented programs--research of an applied nature which is not encompassed by the disease-oriented institutes of NIH.

Following are a few examples of research findings, selected for their interest value and as illustrative of major areas of program activity in the Division during 1960. Some of these findings have implications for clinical medicine which are already apparent; others have implications for the solution of practical problems in environmental health. Most are basic--welcomed within the technical disciplines primarily for their ability to

close gaps in the body of general knowledge which serves as the foundational structure of clinical medicine. The practical usefulness of most of the latter findings can only be assessed after they have been assimilated from the technical literature by investigators and practitioners in medical fields and incorporated into new approaches to research and clinical problems.

BASIC BIOMEDICAL FINDINGS

CLEARER DEFINITION OF THE GENE FROM TEXAS STUDIES IN BREAD MOLD

NIH Grantees at the University of Texas are exploiting the genetic and nutritional simplicity of the red bread mold Neurospora to map the locations, on particular chromosomes, of the determinants of biochemical reactions by which particular substrates are metabolized for utilization in life processes. The work is expected to lead to a clearer definition of the gene.

The red bread mold Neurospora has long been regarded as one of the most useful of genetic experimental organisms. Pure strains can be multiplied a million-fold in a few days without genetic change. Each cell nucleus contains only a single set of genes, so recessive characters are not hidden by dominants, as they are in organisms with the double set, such as man. Neurospora can be grown in pure culture on a chemically known medium containing only sugar and a B vitamin (Biotin) plus a few inorganic substances. In the natural state, the mold possesses the biochemical apparatus to make, from these simple materials, all the other constituents of its cells. Thus when a genetic element is experimentally disrupted by irradiation the mutation may be expressed and mapped in terms of the measurable changes which occur in the simple nutritional requirements of the organism.

A large group of such nutritional mutations of Neurospora is described in Proceedings of the National Academy of Sciences by R. P. Wagner, C. E. Somers and A. Bergquist, at the University of Texas in Austin. Plotting the distribution of the mutations on a map of Neurospora's chromosomes, Wagner's group traced all of them to a limited area of the fifth chromosome. Wagner's report appears to record the first demonstration, in any organism with single or double chromosome complement, of a direct relationship between one small segment of a single chromosome and a particular set of biochemical reactions. (Damage in the segment of chromosome directly affected the transformation of precursors of two important amino acids--isoleucine and valine.)

The results also strongly indicate that there is a linear arrangement of the chromosomal material, with the active elements of it arranged to correspond to the order of sequence of the biochemical reactions it controls.

Further understanding, valuable in genetics, of what genes are and how they work is expected to result from continued study of the Neurospora system.

Wagner's work is supported by the Division of General Medical Sciences and the National Cancer Institute.

BREAD MOLD CELLS SERVE AS LIVING CENTRIFUGE TUBES FOR CYTOCHEMICAL STUDIES

The tough cylindrical cells of Neurospora (red bread mold) tendrils have been found to serve as living ultra microcentrifuge tubes, allowing the constituent organelles and fluids of the cell to be grouped in layers for biochemical analyses without the necessity for disrupting vital interrelationships. (Cells subjected to 50,000 gravities recover, their organelles regroup, and they continue to grow.) This is reported in Experimental Cell Research by Marko Zalokar of Yale.

Zalokar reports that the order of sedimentation, or layering of cell constituents by centrifugal force, in the living cell is almost the direct reverse of that which results when the cells are first homogenized--a conventional cytochemical technique. The pattern of centrifugal distribution of genetically important substances, such as DNA, in relation to the cell organelles, is also different when living, instead of homogenized, cells are used.

Zalokar points out that much of the progress of modern biochemistry has depended upon the development of methods which do not break down major cell components, even though the cell itself has had to be sacrificed. The availability of a method for biochemical studies during life of the constituents common to all living cells should further genetic and immunologic knowledge of the mechanisms of molecular duplication such as protein synthesis, which are thought to be fundamental in cell reproduction and the determination and maintenance of the biological individuality (characteristic structural and functional detail) of each life form.

GENE FOR MOUSE DWARFISM ACTS FIRST ON PITUITARY, NOT ON THE HYPOTHALAMUS

The question has been raised whether the gene that produces dwarfism in anterior pituitary dwarf mice acts on the pituitary directly, or indirectly through the hypothalamus. Experiments in pituitary transplantation provide convincing evidence that the pituitary gland, not the hypothalamus, is the primary site of action of the dwarfism gene.

Since 1929, when the strain was discovered, the pituitary dwarf mouse has been studied extensively for insights into the nature of human endocrine disorders, including human genetic pituitary dwarfism. As in some of the human conditions, the hereditary dwarfism of the mouse is known to involve arrested development of the anterior pituitary structures which secrete growth hormone, and transplants or injections of normal pituitary material are known to promote normal growth in the dwarfs. Although it is obvious from this that the gene for dwarfism acts on the pituitary gland, the possibility exists that the pituitary effect is a secondary one - that some undefined lesion of the hypothalamus (through which nervous control of some pituitary secretions is mediated) underlies the pituitary deficiency.

An answer to the question of which of the two structures is the primary site of action of the gene has been provided by Robert L. Carsner and Edward G. Rennels of the University of Texas Medical Branch, Galveston.

They report in Science that surgical substitution of normal anterior pituitary glands for dwarf pituitaries (between immature littermates) resulted in mice which, though genetic dwarfs, had the appearance and growth rates of normal mice. But substitution of anterior pituitary from dwarf mice in genetically normal littermates resulted in dwarfed mice, despite their possession of the genetically normal hypothalamus. This shows rather clearly that the anterior lobe of the pituitary and not the hypothalamus is the primary site of gene action in the anterior pituitary dwarf mouse.

THEORY OF AGING IMPLIES DEPENDENCE OF SEX RATIO AT BIRTH ON FATHER'S AGE

Leo Szilard, noted nuclear physicist and theoretical biologist at the University of Chicago, developed a general theory of aging applicable to mammals, during his recent tenure as a consultant to the basic research program of NIMH. The theory assumes that whole chromosomes suffer complete loss of function in single random events ("aging hits") during the lifetime of the individual.

On the basis of this theory, Szilard has derived from American life tables the frequency with which such aging hits should occur in the somatic cells in man. The calculated frequency is once every twelve years, on the average.

Assuming this mechanism and frequency of chromosome loss holds for the immature germ cells which give rise to sperm (the

spermatogonia), Szilard reasons that the ratio of boys to girls at birth should decrease with the age of the father.

He consulted the birth data for the U.S. for 1955 to compute the relationship of the ratio of boys and girls to the ages of their fathers. His calculations from this record of actual events indicate a fall did occur, with increasing paternal age, in the ratio of boys to girls at birth. However, he concludes, data based on larger population samples than the 1955 record must be available before it will be possible to draw conclusions relevant to his theory.

Szilard's work on the frequency of aging hits in man and the relationship to sex ratio is published in Nature.

EVIDENCE FOR RNA ROLE IN SYNTHESIS OF ANTIBODIES

Seeking the source of the immune response by which organisms "learn" to recognize and destroy foreign proteins, an Israeli team with DGMS support has gained evidence of antibody production and storage in the protein which occurs in combination with ribonucleic acid (RNA) in the cells of antibody-forming organs.

The nucleic acids DNA and RNA are best known as vehicles for the patterns of life's continuity in genetic reproduction - DNA in the genes as chemical repository of the individual protein codes which are the blueprints of the species, and RNA as the cytoplasmic "template" in which each of the protein constituents of cell, organ, and organism is reproduced in conformity with its particular DNA code.

The minute cytoplasmic particles in which the RNA of protein-producing cells is concentrated were reported earlier by NIAMD grantees in Boston to contain much protein which is bound with the RNA in a chemical complex. Thus the RNA actually occurs in those particles as ribonucleoprotein, and the particles themselves have been termed "ribonucleoprotein particles" by the Boston workers.

The 1960 DGMS-aided work, by a team at the Weizmann Institute in Rehovoth, Israel, supports the idea that the ribonucleoprotein complex of the cell may play a role in the synthesis of antibodies. This concept was suggested earlier by less definitive work done in Czechoslovakia.

Although the Weizmann findings do not permit conclusive definition of the role of ribonucleoprotein in antibody synthesis, the investigators observed that the antibody response in their experimental animals involved a marked increase in the ribonucleoprotein content of the antibody-forming organs and that the

protein part of the ribonucleoprotein complex obtained from these organs contains inactive antibodies which are released in active form on destruction of the ribonucleic acid part of the complex.

The findings accord with the idea that the antibody molecule (which is itself a protein) forms part of the ribonucleoprotein complex of the cell and that this complex is the site of synthesis of antibody, according to the Weizmann investigators.

M. Feldman, D. Elson and A. Globerson of the Weizmann team published the findings in Nature.

ANTIBODY FORMATION AFTER ANTIGEN IS GONE OCCURS FOR MANY OR ALL ANTIGENS

Continuous synthesis of antibody long after a single injection of the antigen, occurred in rabbits in response to bovine serum albumin, guinea pig serum protein, and egg albumin and its azo derivatives. The findings, by Maxwell Richter and Felix Haurowitz of Indiana University, Bloomington, show for the first time that continuous antibody formation long after primary immunization, is stimulated not only by the carbohydrate antigens, toxoids, and viruses, but also by typical innocuous protein antigens.

This has important implications for understanding the secondary response (and associated sensitivity reactions). Failure to detect circulating antibody months after introduction of an antigen, followed by the rapid appearance of precipitating antibody upon a second exposure, is often attributed to "memory" of the antibody-producing cells for the departed antigen (hence the term "anamnestic" reaction for this phenomenon). According to the Bloomington work, the reintroduced antigen, even when given months after the primary immunization, is being given to an organism which is still making the antibody.

In every instance, the Bloomington investigators were able to detect the antibodies in the circulation for months after a single antigen injection -- but not by using the conventional precipitation methods of antibody detection. The presence of the antibodies was demonstrated only by the agglutination of antigen-coated red blood cells. The antibodies were of the non-precipitating type.

"With the application of techniques which do not depend on the precipitability of the antibody it will undoubtedly be demonstrated that maintenance of antibody synthesis for a long period of time after antigen administration is a universal phenomenon and not an isolated observation which depends on the nature of the antigen used," Richter and Haurowitz predict in the Journal of Immunology.

FEMALE SURVIVAL ADVANTAGE EXTENDS TO FIRST WEEKS OF EMBRYONIC LIFE

Sex determination by chromosome count in the placenta is extending knowledge of the sex ratio in human mortality throughout the prenatal period. Evidence thus far indicates that the female advantage over the male in infant survival extends back to the earliest days of pregnancy.

Investigators of the State University of New York, New York City, have used sex chromatin to determine gender in 242 prenatal deaths at from one to 24 weeks of gestation. They found only 93 females and 149 males--a ratio of 100:160.2.

The findings, though preliminary, indicate that it is the male who makes up the greater part of early pregnancy wastage, as he does the mortality of infants over 1,000 grams (the perinatal mortality).

Fresh placental tissue, obtained at the time of currettage for incomplete abortions, was used. Basing judgement of sex on the chromatin of placental, rather than fetal cells, the New York group was able to extend sex determination back to the first week of pregnancy, and make determinations even when the embryo itself was not available.

No correlation was found between sex ratio and gestational age of the miscarried fetuses.

The results may seem surprising by some present lines of reasoning. For example, according to present knowledge of the mechanism by which the paired chromosomes divide as the sex cells mature, there should be as many male as female-determining sperm (half the sperms are X-bearing and half Y-bearing). Thus, one may expect a sex ratio of 1 to 1 at the time of fertilization. Since the accepted sex ratio at birth the world over is about 106 males to 100 females, one might expect that more females than males die in the earliest months of pregnancy.

This has been one of the speculations--there was no way to determining the reality until Barr's recent discovery of the microscopically visible sex difference in the chromosomes, and the present accumulation of data. The data thus far suggest that the sex ratio in man at the time of fertilization may be about 111 to 100, male to female. More data is needed before the true primary sex ratio can be established with certainty.

Vincent Tricomi, David Serr and George Solish published the findings in the American Journal of Obstetrics and Gynecology.

NORMAL DEVELOPMENT OF PUBIC SYMPHYSIS IN ABNORMAL LOCATION

Evidence that the pubic symphysis can develop normally when transplanted out of its normal site, and that the transplant retains its ability to respond specifically to estrogen and relaxin at maturity, is published by E. S. Crelin of Yale, in the Anatomical Record.

The findings indicate that the ability of the symphysis to respond to these hormones at the time of life when they are active (sexual maturity, pregnancy and delivery) is innate in the tissues of this pelvic joint ("...predetermined by the immature tissue from which the symphysis develops").

Crelin reports that the pubic symphysis (including its cartilage and the angles of the pubic bones) was transplanted into the muscle of the chest (pectoralis muscle) in 50 immature (12 day) mice. Half of the mice received injections of estrogen and of a relaxin preparation at four-day intervals for 18 days prior to the time the transplants were finally examined at eight weeks of age.

With few exceptions, he reports, gross and histologic examinations of the transplants of the untreated mice showed they had developed into pubic symphyses essentially identical to those of normal virgin adults. The transplants of the hormone-treated mice had undergone the normal interpubic transformation specific for such pelvic tissues (resorption of bone in response to estrogen, and changes in the ligaments, related to their normal relaxation during pregnancy and delivery).

ADVANCE IN CHROMATOGRAPHY MAY AID RESEARCH ON ROLES OF MAJOR BIOGENIC AMINES

Jorge Awapara, Virginia Davis and Odette Graham of the Rice Institute, Houston, Texas, report the development of column chromatographic methods enabling complete separation of a number of naturally occurring amines from their parent amino acids and from some of their derivatives.

Thus a step has been taken toward elimination of one of the difficulties in detecting small amounts of amines in tissues--the fact that they are indistinguishable from their parent amino acids by many of the known tests. Using known mixtures of various amino acids and amines in comparing conventional testing methods with their own innovations, the Houston workers achieved the desired separation and quantitation by column chromatography in which the stationary phase was cellulose and the mobile phase a mixture of butanol-acetic acid-water. They tested the method

in the identification of hordenine in germinating barley (where it accumulates in the roots in the first week of life), and found large amounts of hordenine and eight other amines as well.

The part played by amines in life processes has been attracting the interest of investigators in virtually every major field of biologic science. Many drugs are believed to exert effects by influencing amine metabolism. In most animals and bacteria, amines appear to be made from their parent amino acids by decarboxylation. In this connection, it may be of interest that the Rice Institute workers suggest that a relatively easy method for measuring amino acid decarboxylases may be developed as an extension of their reported work.

Their work is reported in the Journal of Chromatography.

OXYGEN-CARRYING CAPACITY OF RESPIRATORY PIGMENTS DEMONSTRATED EXPERIMENTALLY

In vitro experiments by P. F. Scholander at Scripps Institution of Oceanography, La Jolla, California, have indicated that under steady-state conditions and at low tension, oxygen may be transported as much as eight times as fast by a solution of human hemoglobin as by water. Increasing the pressure one atmosphere has the effect of doubling the rate of transport. The same effect occurs in intact red cells and in myoglobin, the respiratory pigment of muscle.

The transfer of oxygen to the cells of the body is essential for the maintenance of life. The complexities surrounding the manner in which this transport is carried out have not been fully understood, however. It is clear that passive diffusion is a relatively slow process and that oxygen has a low solubility in water. The oxygen-carrying property of hemoglobin is the vital process which makes adequate tissue oxygenation possible. Among the pigments with oxygen-binding capacity, the hemoglobin of the red cell and the myoglobin of the muscle are most abundant in the body. Hemoglobin is transported rapidly to every part of the body, while myoglobin is confined within the muscle system and is stationary.

In his paper published in Science, Scholander has proposed that the system for transport of molecules resembles a conveyor belt or "bucket brigade" in which oxygen molecules are handed down from one to the next, in a chain. The idea is postulated for both hemoglobin and myoglobin.

FETAL HEMOGLOBIN MAY BE AN ADAPTATION TO DEFICIENCIES IN THE FETAL ENVIRONMENT

It is not known what causes the gradual decrease of the fetal form of hemoglobin in normally maturing infants, or the persistence of fetal hemoglobin in adults with certain inborn anemias, such as thalassemia. Some light may be thrown on the underlying mechanisms as a result of studies in Boston, Massachusetts, showing influences of cellular environment on the relative quantities of fetal and adult hemoglobin synthesized by human immature red cells (reticulocytes).

David W. Allen and James H. Jandl used isotopic labelling techniques in in vitro measurements of fetal and adult hemoglobin formed in reticulocyte-rich suspensions of umbilical cord blood drawn at the time of delivery. They found that deficiencies of oxygen and glucose favored the production of the fetal hemoglobin compared with the adult type in the suspensions.

This raises the possibility that the fetal hemoglobin is an adaptation to the environment of the fetus, wherein oxygen and glucose are at a premium, Allen and Jandl conclude. The possibility provides impetus for inquiry into defects of energy metabolism in congenital anemias of adults which are characterized by the persistence of large amounts of fetal hemoglobin, they suggested.

These findings are reported in The Journal of Clinical Investigation. Following submission of this report, similar observations agreeing with those of Allen's group were published by another research group (E.D. Thomas, et al) in the British journal Nature.

The Boston work is reported from the Thorndike Memorial Laboratory, Boston City Hospital, and Harvard Medical School.

RED CELL GENERATING AGENT OF "ALTITUDE GOAT" MILK FOUND ACTIVE IN RATS

An erythropoietic (red blood cell-generating) factor seems to occur in the milk of lactating goats exposed four hours daily to an altitude of 22,000 feet (simulated by decompression chamber). Evidence tentatively suggesting existence of the "erythropoietin" was published in The Physiologist in 1958, by J. Clifford Stickney and co-workers at West Virginia University in Morgantown. The West Virginia group reported then that four kids, maintained from birth on the milk of such "altitude goats," showed slightly higher hemoglobin levels and immature red cell counts than did four control kids fed milk from goats kept at ground level. The West Virginia group reports in 1960 (Federation Proceedings) that extracts of the milk whey of three such "altitude goats" raised

hemoglobin levels and reticulocyte (immature red cell) counts when injected into the peritoneal cavity in rats. These blood changes did not occur in control rats injected with whey material obtained by the same extraction method from the same three goats when they had not been subjected to the decompression and hypoxia of the "altitude" treatment. Thus the activity of the erythropoietin in the "altitude goat" milk does not appear to be limited to the goat.

The role (or roles) of the erythropoietins in influencing red cell production in normal and disease states is poorly understood. Additional knowledge of erythropoietin origins and activities is valued for the light it may throw on mechanisms of hemoglobin and red cell production. This understanding could help solve practical problems of red cell and hemoglobin inadequacy in situations of tissue oxygen deprivation due to disease (as in anemias) and of atmospheric oxygen rarity (as in high altitude flying).

The 1960 work was published by Stickney, Theadore L. Browne and Edward J. Val Liere.

NEW CENTRIFUGE FOR STUDY OF GRAVITY IN CONTROL OF MAMMALIAN GROWTH

A multifield centrifuge capable of holding cages large enough for small mammals to be maintained under continuous high gravity for years is announced from the State University of Iowa, Iowa City. No such centrifuge had been previously available commercially, and little experimental data have been available on the role of gravity as an environmental factor in the control of mammalian growth.

The centrifuge has been in continuous operation for over 18 months. Mice have been reared in it under high gravity conditions; and it is now being prepared for use with kittens.

The apparatus is described and illustrated by Gerald Walters, Charles C. Wunder and Lincoln Smith in the Journal of Applied Physiology.

THIGH BONES SHOW STRIKING GROWTH CHANGES AT FOUR TIMES EARTH GRAVITY

Thigh bones of mice reared at four times the earth's gravity grow faster than other body tissues and assume a more circular cross-section, according to a study at the State University of Iowa, Iowa City. Although the explanation of this is not definitely known, the circular cross-section would give this supporting structure more strength with which to resist gravity.

The shape and size of body parts are believed to be influenced by gravity, although little data has been available on the role of this environmental factor in growth processes.

Having reared mice at four centrifugal gravities, the Iowa investigators report that the thigh bone effects of the gravity in female mice were more striking than any other effects. At first the experimental animals fell behind the controls in body mass, then continued to grow. But the femurs grew continously from the start as fast as or faster than those of the controls, acquiring a more circular cross-section.

Further work is necessary before actual causes of the bone changes are known, but the circular form would give the supporting structure more strength against gravity. Wolff's law proposes that mechanical force will evoke growth of the stressed part of the bone, and the artificial multiplication of earth's gravity in the experiments would certainly cause such stresses.

The observed general loss of body mass is attributed by the Iowa City workers to lowered food consumption during the first week in the centrifuge.

The data is published in Nature by Charles C. Wunder, Stanley R. Briney, Melvin Kral and Charles Skaugstad.

ACOUSTIC AND RESPIRATORY SIGNIFICANCE OF GEOMETRIC CONFIGURATION OF LARYNX

B. Raymond Fink of The Presbyterian Hospital and Columbia University, New York City, and F. Kirschner of the General Electric Company, Bloomfield, New Jersey, in studies of the geometrical configuration of the larynx with X-rays, have observed that the cavity below the vocal apparatus forms an aerodynamic nozzle whose mouth looks into the trachea while the throat accelerates the flow toward the oral cavity. Respiratory air flowing through the larynx traverses a manifold section of two horns formed by the vocal folds and the vestibular folds. The system allows optimum flow during breathing. The research is reported in the International Journal of Phoniatry.

The investigators studied 11 women and 12 men, four of whom had unilateral laryngeal nerve paralysis and one, bilateral paralysis. The use of X-rays, which were taken during slow expiration and phonation, made it possible to employ the living larynx as a model and to observe its configuration directly, without interference from instruments.

It was found that contraction of the vocal muscle within the vocal folds during expiration, inspiration, and speech, maintains

the contour of an exponential nozzle between the vocal folds. The surface of the folds is convex, with an approximately exponential curvature, which has been shown to produce the lowest coefficient of resistance to airflow. In paralysis the vocal folds are prolapsed and their surface is concave. The values for the coefficient of normal flow resistance were computed from the hydrodynamic equation of flow and verified by direct measurements on models of five different types of acoustical connectors. The lowest resistance was obtained from the model fashioned after the human exponential connector.

The investigators found that there was acoustical significance in their study in that the exponential horn in the larynx serves as an acoustic transformer between the infralaryngeal and supralaryngeal cavaties. It was of interest that the subject with the best trained voice in the studies also had the smoothest horn in the larynx.

The findings demonstrate that increased respiratory effort is required in cases of laryngeal paralysis, on the basis of the inefficient contour produced as well as the decrease in the diameter of the orifices.

PLACENTAL FOCUS OF UTERINE MUSCLE CONTROL SHOWN BY SUBSTITUTING WAX FOR FETUS

Despite the experimental work of past decades establishing the role of progesterone in the maintenance of mammalian pregnancy, repeated failure of progesterone therapy to prevent abortive uterine contractions led many obstetricians in recent years to doubt the significance of this ovarian hormone in the maintenance of human pregnancy.

The inconsistent uterine effects of systemic progesterone were partly explained by the recent experimental studies of Arpad Csapo and others, indicating that the myometrial effect of progesterone is a local one that declines with increasing distance from the placenta. Csapo postulated that the level of progesterone in the general circulation is not of significance in the birth process, but that the local uterine concentration of the hormone--its diffusion from the placenta--is of major importance.

A research team at the Roscoe B. Jackson Memorial Laboratory, Bar Harbor. Maine, illustrated this placental localization of uterine muscle activity in rabbits by insertions of straight paraffin rods into the uterine lumen following removal of the fetuses (but not their placentas). Within 48 hours, marked curvatures and constrictions appeared in the rods. The constrictions occurred at points midway between adjacent placentas. The degree of rod curvature was related to the distance of the rod from the placenta.

"Since progesterone acts to dampen uterine contractions," the Bar Harbor group reports in Fertility and Sterility, "it is clear that the muscle closest to the placenta is under a greater degree of progesterone control than the muscle furthest from the placenta. In this respect, these results are in harmony with the work of Csapo on the concept of a local gradient in the progesterone level in the uterine musculature."

The Bar Harbor studies were supported by DGMS and NIMH research grants. M. X. Zarrow, E.D. Wilson, A. Lee Caldwell, Jr., J. Yochim and P. B. Sawin participated in the work.

FINDINGS PERTINENT TO ENVIRONMENTAL HEALTH

CYANIDE PROBLEM IN WATER POLLUTION MAY YIELD TO BIOLOGICAL TREATMENT

Using bacterial cultures (activated sludge systems) like the ones used for sewage treatment, studies in Lancaster, Pennsylvania, have shown that inorganic cyanide can be inactivated by biological treatment without the further addition of sewage, the organisms coming to rely exclusively on the cyanide for their carbon and nitrogen nutritional requirements.

The inorganic cyanide wastes produced in metal plating and hardening processes are said to be among the most toxic wastes produced in industry. Not only are small quantities toxic to fish, but even smaller quantities may interrupt the natural purification processes of streams by killing off the responsible organisms. The same thing generally happens when cyanide gets into the biological treatment systems of sewage disposal plants.

Most present day cyanide disposal methods are chemical processes expensive in their requirements for heat, electric current, chemicals, and the further disposal of such by-products as toxic hydrogen cyanide gas.

Reporting in the Journal of the Sanitary Engineering Division, Proceedings of the American Society of Civil Engineers, the Pennsylvania group credits the British Water Pollution Research Laboratory for most of the research done to date on the biologic inactivation of inorganic cyanide. The British workers had subjected complex metal cyanides to a continuing feed of sewage in trickling filters, with a view to subjecting the cyanide to the same complex of natural biologic and chemical activities which work so usefully on sewage. This was effective in demonstrating what the Lancaster group refer to as "the extreme versatility of a biologic system in the treatment of industrial wastes," and resulted in the construction of a few experimental sewage-feed trickling filter plants for treating cyanide wastes.

The British work convinced the American engineers that the inorganic cyanides were amenable to biologic treatment methods which would be more economical than the present chemical ones. The purpose of their laboratory work at Pennsylvania State University in Lancaster was to gain basic metabolic data on the microbiologic processes and to learn if the cyanide can be metabolized without feeding sewage into the system. They began with a culture of sewage organisms (an activated sludge from a college sewage treatment plant) as the source of the richly varied bacterial flora they would need. They acclimated the sludge by gradually substituting cyanide and nutrients, until a flourishing culture was obtained which was relying on cyanide exclusively for its carbon and nitrogen (no more sewage had to be fed).

One of the systems they developed metabolized over 99% of the cyanide, converting 97.9% of the cyanide carbon to harmless CO_2 gas, and 90% of the cyanide nitrogen to ammonia, nitrite, and nitrate.

Authors of the study were John Nesbitt of the Pennsylvania State University in Lancaster, Robert Kohl from Gilbert Associates, Cleveland, Ohio, and Elmer Wagner of Huth Engineers in Lancaster.

STERILITY IN LEAD-EXPOSED WOMEN EXPLAINED BY STUDY OF EFFECT ON MONKEY OVARY

The damaging effects of lead on female reproductive functions have given rise to laws protecting women in occupations with lead hazards. Women exposed to lead show disturbances of menstruation, and periods of sterility. If pregnancy does occur, the chances of miscarriage are increased. Children born are apt to be weak, sickly, and slow to develop. Those who survive may be mentally retarded.

Gertrude J. Vermande-Van Eck and J. Wister Meigs at Yale University, New Haven, Connecticut, chose the Rhesus monkey for studies of the effects of lead on the reproductive system. The similarity of human and Rhesus ovaries and of the cycle of ovulation (the 28 day duration, with one egg per cycle ovulated on day 11-16, and the similar cyclic bleeding and vaginal changes) suggested that results in the monkey would have significance for humans.

With weekly injections of lead chloride, the same clinical and laboratory symptoms developed in all eleven monkeys that are commonly seen in human industrial lead intoxication. Urinary lead rose very high, beginning within 48 hours. The familiar "lead line" of bluish-gray lead sulfide deposits on the gums appeared after three or four months. Weight dropped, hypochromic anemia developed, and one of the eleven developed neurologic symptoms (paralysis).

The menstrual periods ceased after an average of two months and virtually all signs of other ovarian influences (in sex skin color and swelling) had disappeared after eight months of injections.

One ovary was removed from each monkey and examined at eight months (the peak of lead intoxication). These ovaries were abnormally flat and white, showing no ripening egg follicles. Microscopic examination showed degenerating germ cells and encroachment of connective tissue throughout the organ.

The monkeys were then allowed to recover, and after eight months without lead injections, the other ovary was removed. It was found normal, both grossly and microscopically, with normally developing egg follicles. Recovery was general when the lead injections were stopped. Gum lead line disappeared, anemia improved rapidly, weight increased, and normal menstrual periods and other gonadal functions returned in one to five months. Microscopic examination of kidneys showed very little evidence of damage at the end of the study.

One interesting finding was that orally administered lead did not seem to be absorbed. None appeared in the urine and no symptoms of intoxication developed from it.

Regarding the ovarian changes, the investigators summarize, in Fertility and Sterility; "The results support clinical observations of menstrual abnormalities in women exposed to lead. It was evident that our animals were unable to conceive, and it is likely that the sterility found in lead-exposed women has a similar origin."

URINARY COPROPORPHYRIN TEST FOR SCREENING LEAD POISONING SUSPECTS

A simple qualitative technique for rapid clinical urine coproporphyrin estimation, has proven reliable and sensitive as a screening test in 258 Baltimore children in whom lead poisoning was suspected. The chief value of the test is its ability to disclose those patients in whom brain damage may be imminent from large amounts of ingested lead, but who do not give a clear diagnostic picture of lead poisoning. The test was uniformly positive in such patients in the Baltimore series, whereas other quickly available ancillary methods were not.

This is reported from the Johns Hopkins University School of Medicine and Hospital, and the Baltimore City Hospitals, Baltimore. Philip F. Benson, now at Guy's Hospital, London, and J. Julian Chisolm, Jr. of the Baltimore City Hospitals, report the technique and the results of its laboratory and clinical evaluation, in the Journal of Pediatrics.

Derangement of porphyrin metabolism, associated with increased urinary excretion of coproporphyrin, is an early and constant finding in lead intoxication. Early diagnosis and treatment are important for preventing the brain lesions of severe lead poisoning and the consequent high risk of neurologic disorders and death.

The technique involves extraction of the urine in ether and hydrochloric acid. The coproporphyrin concentration of the sample tube is estimated by its fluorescence intensity as compared with that of a standard tube. Results are expressed on an arbitrary scale ranging from 0 to ++++ (as 0 or + for normal, ++ for slight elevation, and +++ or ++++ for marked elevation indicating dangerous amounts of lead in the tissues). The technique is said to be easily acquired and to take only a few minutes. The apparatus and reagents are readily available to physicians.

FIND ROLE FOR SYMPATHETIC NERVOUS SYSTEM IN CARBON TETRACHLORIDE TOXICITY

The characteristic liver damage of carbon tetrachloride poisoning may result, not from direct injury to the tissues, but indirectly from their oxygen starvation. Evidence is presented that this oxygen deprivation is caused by stimulation of the central sympathetic nervous system, leading to restriction of blood flow to the liver. These are results of studies by Theodore M. Brody, Deane N. Calvert and Kenneth E. Moore at the University of Michigan, Ann Arbor.

The liver damage of excessive exposure to carbon tetrachloride and related halogenated hydrocarbons has been a major cause of industrial poisoning, and the mechanism of the poisoning has been a subject of extensive research for many years. Two very different concepts of the mechanism of toxicity have arisen. One holds that the chemical produces the damage indirectly by causing swelling of liver cells, cutting off the tissue oxygen supply. The other more recent line of reasoning suggests that it directly attacks the mitochondria of the liver cell, leading to loss of enzyme activity vital to the tissues.

The Michigan investigators present evidence that carbon tetrachloride and related chemicals may produce the liver damage indirectly by acting on the central nervous system with a prolonged rise in central sympathetic outflow reinforced by epinephrine release from the adrenal.

They report that experimental stimulation of sympathetic nervous supply to the liver can cause restriction of blood flow and tissue damage in the liver. Further, stimulation of the sympathetic nerve supply to fat depots causes a release of unesterified

fatty acids and subsequent fatty deposition in the liver, as occurs in carbon tetrachloride poisoning. Conversely, sectioning of the spinal cord, adrenergic blockage, and other maneuvers to modify the effects of sympathetic stimulation, can modify some of the liver changes typical of toxic dosage of carbon tetrachloride in the rat.

These and other findings implicating the sympathetic nervous system are reported in three papers in the American Journal of Physiology.

TROUT ACCUMULATE CHROMIUM CONTAMINATING THEIR WATERS AT SUBLETHAL LEVELS

Although chromium contamination of water is known to be toxic to important food and game fish, past fish studies have been limited to the determination of lethal doses, and little is known of the effects of exposure to sublethal levels. From such a study in rainbow trout, J. Knoll and P. O. Fromm of Michigan State University in East Lansing report that these fish can accumulate chromium in several organs to levels above the environment when exposed to 2.5 milligrams per liter of water, a relatively low level of environmental contamination.

Chromium is of long range significance as a pollutant of streams, lakes and potable water supplies, due to waste disposal by industry. Considerable amounts of the metal in its hexavalent form are often present in the environment of fishes.

The chromium accumulation measured in the trout by the Michigan investigators occurred in the spleen, posterior gut, pyloric caeca, stomach, liver, and kidney. The amount accumulated in muscle, the edible part of the fish, was insignificant, indicating that human consumption of rainbow trout taken from waters contaminated with chromium at levels no greater than 2.5 milligrams per liter would probably present no serious human health hazard.

The Michigan study was reported in Physiological Zoology.

TOXIC NITROGEN DIOXIDE GAS IN FARM SILOS AS EXTENSIVE HEALTH HAZARD FOR FARMERS

The occurrence of nitrogen dioxide gas in farm silos has only been reported within the last decade, and has been increasingly implicated in deaths and serious incapacitation among farmers. It is now regarded as a definite farm occupational hazard.

The seriousness of the hazard has been indicated by a DGMS-aided statewide survey in Minnesota. As part of the survey, 332 individual farm silos, and factors associated with the preparation

of their silage, were subjected to detailed study by J. V. Scaletti, C. E. Gates, R. A. Briggs and L. M. Schumann of the University of Minnesota, St. Paul.

Forty-two percent of the silos showed concentrations of nitrogen dioxide considered to be hazardous (exceeding 4 parts per million of air). Studies of related agronomic practices and conditions showed that the greater the amount of organic matter in the farm soils at the time the silage crop was prepared, the greater the number of positive tests for the gas in silo air. Significant, though weaker, correlations with levels of available potassium and phosphorus in the soil were also recorded.

Use of sodium metabisulfate as a silage preservative seemed to be associated with a reduced occurrence of the hazard. (Only one of 21 silos containing this chemical showed evidence of nitrogen dioxide production.)

The exact relationship of the gas production to the factors found associated with it is not yet known--the study was preliminary and exploratory. The findings are published in the Agronomy Journal.

PATTERNS OF CHILD INJURY IN CAR ACCIDENTS REPORTED FROM STUDY AT CORNELL

A biostatistical study by Cornell University of the measurable consequences of automobile accidents has revealed a pattern of injury for exposed children which differs from that for exposed adolescents and adults. Fifty-two percent of exposed children (under 12 years) were hurt, compared with 71 percent of exposed adolescents (12-18 years) and 80 percent of adults. The multiplicity of injuries also increases with age, children averaging 1.4 body areas involved, adolescents 2.0, and adults 2.2. Head injuries were more frequent in children, injuries to other body areas less frequent.

The injuries are milder for children when they occur in the head, neck, thorax, and abdomen. Their lower extremity injuries are more severe.

In each seating area of the car, injury increases with increasing age. In each age group, injury associates with seat area occupied; rear seats are safest (frequency and severity of injury are lowest). "Unusual" seating appeared to be more dangerous than either front or rear seating in the usual arrangements. ("Unusual" is an omnibus category including persons standing, reclining, lap-carried, fourth occupants of three-occupant seats, occupants of center-bank seats in station wagons, etc.)

The data of this study were drawn exclusively from non-pedestrian accidents of passenger cars, occurring on rural and open highways, and involving injury to at least one occupant. Altogether, 14,520 cars, 31,925 exposed occupants and 23,475 injured occupants of known age were considered in the study.

The Automotive Crash Injury Research of Cornell University, New York City, is sponsored by research contracts with the Department of the Army, supplemented by grants from the NIH Division of General Medical Sciences. The data were collected by John O. Moore, Boris Tourin, John W. Garrett and Robert Lilienfeld of the Cornell staff, with the collaboration of physicians, public health workers, and investigating officers working in the states.

The results of the study on child injuries were announced in part during 1959 at the 14th International Conference on Pediatrics, Montreal, Canada, and at the Annual Convention of the Florida Society for Crippled Children, Palm Beach, Florida. A paper describing the completed work was made available to DGMS in 1960.

FINDINGS PERTINENT TO CLINICAL MEDICINE

ADVANTAGES OF SALINE OVER BLOOD FOR REPLACEMENT OF FLUID IN SHOCK FROM BURNS

Impressed by the burden of residual effects of present burn treatment methods, Ben Wilson and Jerry Stirman of Southwestern Medical School, Dallas, Texas, have sought modes of burn treatment which are comparable in simplicity and reversibility to the derangements they are intended to remedy. They emphasize that the early life-threatening tendency to acute fluid volume loss and circulatory failure (shock) in burns is a passing phase, and that a mode of treatment should be used which compensates this loss only during the time it threatens recovery. Thus the preferred medium for expanding diminished fluid volume in burn shock would be solutions of physiologic salts, given orally or intravenously, rather than transfusions of whole blood, which has effects lasting beyond the transient crisis of shock.

The evidence reinforcing this philosophy was obtained in an analysis of records of 430 patients, treated for burns within 48 hours, with or without the use of whole blood. The reported findings seem to confirm that the simplest and safest available modality for routine treatment of burn shock is saline resembling the blood in salt composition, taken by mouth according to the need expressed by the patient.

The chief complications which prevented satisfactory oral voluntary use of saline in the Dallas study were inability of the

patient to communicate and cooperate (as in unconsciousness), acute stomach dilatation (which contributes its own component of circulatory collapse to that of the burn shock), and acutely elevated body temperature (which also adds separately to circulatory collapse, especially when compression bandages are used in hot weather). The Dallas investigators suggest that under these circumstances, saline be administered intravenously as necessary to make up the loss, and attempts be made to relieve the complicating conditions. (Stomach dilatation is successfully relieved by stomach tube, and overheating by ice packs.)

The investigators acknowledge the existence of circumstances, uncommon in their study population, in which the use of other volume expanders than saline may be preferable in treating burn shock. Infancy, and the severe protein depletion of starvation were mentioned as such circumstances.

For the Dallas study population, cases of first degree burn, and burn involving less than 15% of the body were excluded from the data, because these cases were not considered to require replacement of fluid volume. Cases with more than 65% of the body surface burned, and those over 61 years of age were also excluded, because their expected mortality approached 100%, according to the analytical method they applied to the data ("Bull and Squire's probit analysis").

When cases from the study population were grouped according to the two modes of treatment (volume replacement by saline only, versus volume replacement by whole blood plus saline) and according to percentages of body surface burned, the data showed that the deaths which occurred within the first 10 days were limited entirely to the group which had received whole blood. Analysis of mortality data for all the subjects whose burns covered 30-44% of the body surface showed a 27% mortality rate for those receiving whole blood during their initial treatment, and only 4% for those who had received only saline. For those with 45-64% involvement of body surface, an 89% mortality is reported for those treated with whole blood, while the "saline only" group showed a mortality rate of 36%. This difference is described as highly significant, indicating that a balanced salt solution can be used effectively and safely in extensive burns as well as those involving only limited areas of the body.

A similar experience with mortality difference for the two kinds of therapy was found when mortality was correlated with depth of burn, as well as extent.

Experimental and clinical studies indicating cause of death (usually respiratory failure) following inadequate treatment of burn shock are also presented by the Dallas team. Other treatment formulae, derived from their experience and philosophy of

burn management are also suggested by the investigators in their report in The Journal of the American Medical Association. Detail as to preparation and administration of salt solutions for oral and intravenous use; and suggestions concerning management of exceptional cases, pain, contractures, and wound debridement and care are included.

FETAL ECG'S ENABLE INBORN HEART DISEASE DIAGNOSIS BEFORE BIRTH

Saul D. Larks and co-workers at the University of California have shown in the past two years that the fetal electrocardiogram (ECG) becomes large and clear at about the 20th week of pregnancy, and that it is useful for the diagnosis of multiple pregnancies.

Larks, and Lawrence Longo, now report in The Journal of the American Medical Association, recognition of congenital heart disease before birth in four infants by this method. The ECG's showed three of the infants to have interrupted conduction of heartbeat from atria to ventricle (heart block). In the fourth, abnormal prenatal ECG was shown at death (five days after delivery) to be due to a coarctation, or constriction, of the aorta.

Prenatal diagnosis could prove a life saving advance in diagnostic ability; for some of the cases may be correctable surgically. It may also be possible, with the aid of the fetal ECG, to determine precisely when the congenital malformations arise and thus to relate their occurrence to suspected causes, such as maternal infections.

URGE CONCEPT OF ESSENTIAL FATTY ACIDS AS FOOD TO BE INCLUDED IN BALANCED DIET

Public and professional interest has centered for some time on the role of the saturated and unsaturated dietary fatty acids in health and disease, especially regarding the diverse effects of these two groups of dietary lipids on the blood lipids, in connection with atherosclerosis.

The suggestion now has been made that the essential unsaturated fatty acids have important functions in fat transport, that excessive feeding of saturated fat may lead to deficiency of the essential unsaturated, and that the concept of a balanced diet should be enlarged to include consideration of the balance of essential versus nonessential fatty acids.

"In the interests of preventive medicine, a plea is made for the nutritional rather than therapeutic approach to the problem of providing sufficient essential fatty acids for maintenance of normal health. That is, essential fatty acids should be considered food rather than medicine."

These are the concluding lines of a paper on "Essential Fatty Acids in Nutrition and Metabolism" by Ralph T. Holman in the A.M.A. Archives of Internal Medicine. Holman is Professor of Physiological Chemistry at the Hormel Institute, University of Minnesota, Austin, Minnesota.

From one line of investigation Holman learned that certain tissues (heart and testis) show dramatic changes in fatty acid constitution on an EFA deficient diet, and that those changes can be made to occur much more quickly and drastically (at least in testis) if any of several techniques for raising the blood lipids are applied simultaneously. Depletion of the polyunsaturated fatty acids of the tissues to the point of their actual degeneration was possible only by the combining of dietary cholesterol or hydrogenated fat with EFA deficiency.

"These results suggest that influences which elevate plasma cholesterol deplete the animal of his store of polyunsaturated fatty acids and thereby hasten EFA deficiency," he summarizes. "If this be the case, then polyunsaturated fatty acids (including EFA) are necessary for the normal transport of cholesterol and plasma lipids. This function of EFA probably would require much larger amounts of EFA than does the maintenance of normal tissue without the stress of excessive lipid transport."

Holman also reports studies showing that "despite radical changes in dietary fat, the cholesterol and polyunsaturated acid contents of the plasma remained rather constant." He interprets this to mean that the EFA required for fat transport in the blood tend to be maintained in the blood even at the cost of the EFA in the tissues. "Prolonged feeding of a saturated fat could therefore lead to a depletion of stores of unsaturated acids and a chronic EFA deficiency," he summarizes.

Evidence of a fundamental need within the cell itself for adequate and balanced supply of these unsaturated fatty acids is presented from studies at the level of enzymatically active subcellular particles, by Holman and others. Evidence is included of the existence of large proportions of the polyunsaturated acids in the enzymatically active particles of beef heart, and of rapid depletion of these substances in subcellular particles when a fat deficient diet is fed. "The changes in polyunsaturated acid composition takes place several weeks before deficiency symptoms appear, suggesting that a primary metabolic lesion is the first result of deficiency," Holman explains. The evidence suggests the easily oxidized unsaturated and essential fatty acids may function as active centers in enzyme structures important in actively metabolizing tissues.

"The essential fatty acids are found abundantly in nature and can readily be obtained in foods on the market," he suggests.

"A wise selection of foods for a balanced diet will provide adequate amounts of essential fatty acids. However, the concept of the balanced diet must be enlarged to include consideration of the balance of essential versus nonessential fatty acids."

CORNELL TEAM, DIVISION OF INDIAN HEALTH AND DGMS IN STUDY OF NAVAJO MEDICINE

A joint medical-anthropological research program on the health of the Navajo is being carried out by Cornell University investigators. They are studying the patterns and socio-economic aspects of disease, the training of Navajos as medical aides, and relationships between physicians and medicine men.

Walsh McDermott, Kurt Deuschle, John Adair, Hugh Fulmer and Bernice Loughlin of the New York Hospital, Cornell University Medical Center, New York City, are conducting the study in cooperation with the Division of Indian Health, PHS. The broad cross-cultural program of work was begun in 1956 to investigate the influence of new types of medical technology. Preliminary findings have been reported in two consecutive issues of Science and in The American Review of Respiratory Diseases. The Navajo Tribal Council also shares in the financial support, which derives partly from a DGMS research grant.

The total program of the Cornell project consists of three large general studies and a number of smaller, segmental, and more sharply defined studies.

The first of the three is a socio-economic study of the community as a whole, with special attention being given to factors pertaining to health and disease. This has brought out the particularly high birth rate among the Navajo, a finding which has bearing on estimating future health and educational needs. In a three-year period, the net gain in population in the group of approximately 2,000 was 239 persons.

The second general study concerns defining the pattern of disease in the community. Complete physical examinations and laboratory studies were carried out. In the first 1,600 persons examined, 76 percent of the disease present was microbial in origin. There were also cases of trachoma, and congenital hip disease was extraordinarily prevalent. Although tuberculosis was fairly widespread, the incidence has fallen substantially during the three-year period. Streptococcal infections and measles appeared to be more serious in Navajo society than in non-Indian.

The third study involves the training of Navajo men and women with limited schooling as field auxiliaries to the public health

nurses. Known as health visitors, these subprofessional workers help the field nurse and often serve as driver-interpreters, sanitarians, and community workers. Three or four health visitors generally assist each public health nurse. The social scientists of the project are studying the relationships between the Navajo health visitors and the community and between the health visitors and the professional staff.

An example of an unexpected cultural barrier faced by the Cornell scientists was the fact that in Navajo society, congenital hip disease is considered neither a disease nor a particular disability. Surgical fusion of the hip joint, as practiced in children in non-Indian society for prevention of a more serious handicap in years to come, complicates the life of the Navajo child for he is then unable to sit easily on the floor or to ride horseback. Rather than improving his lot, modern surgery, in such instances offers no obvious advantages. The Cornell staff is making a genealogic study of the disease, hoping to establish in which families it has existed in the past. Diagnostic X-rays will then be used on infants born to those families, so that the disease will be caught early enough and surgery can thus be avoided.

In one of the segmental studies, Dr. McDermott and his staff are seeking to learn the extent to which an uneducated nonliterate people can assume responsibility in daily self-administration of drugs, such as is necessary in treating tuberculosis. It has been found that approximately 15 percent of the group are unreliable on this score.

In other studies, careful translation of medical and disease concepts is under way. In this regard, social scientists are studying the relationships between physicians and the Navajo medicine men. These relations have been found good, and the medicine men recently have been referring some of their patients to the physicians for advice and treatment.

The Cornell investigators point out that the introduction of any new technology into relatively primitive societies must be undertaken with caution. They are attempting to determine the rate at which new medical concepts can be introduced into the Navajo culture.

EVIDENCE OPPOSES ROUTINE WHOLE BLOOD TRANSFUSIONS DURING GASTRIC OPERATIONS

Opposition to widely advocated practices of routine and prophylactic transfusion of whole blood, particularly during gastric surgery, is expressed in a report by surgeons at the University of Texas, Dallas, Texas. Drawing on a DGMS-aided study

in patients undergoing surgery for benign gastric diseases, they conclude that "routine whole blood transfusions during gastric operations are not necessary."

Ben J. Wilson, and Kenneth O. Adwan present a variety of experimental and clinical evidence in opposition to the following generalizations concerning blood transfusion, which are commonly found in the literature and thought to be reflected in the practice of surgery.

> 1. That knowledge of blood loss during operations, as available in the literature, offers a practical basis for planned transfusions during operation.
>
> 2. That circulatory collapse often accompanies major operations and whole blood should be replaced during operation because it is much simpler to prevent shock than to treat it after it has occurred.
>
> 3. That giving blood to a person in anticipation of subsequent hemorrhage will protect him from the effects of that hemorrhage.
>
> 4. That failure to replace blood loss retards the patient's convalescence, and that replacement is more effective when whole blood is given as the loss occurs.
>
> 5. That the loss of oxygen-carrying red cells that accompanies blood volume loss imposes a burden on recovery that cannot be effectively compensated by replacement with any non-red-cell containing fluid.

The detail of the evidence presented in refutation of each of these assumptions may be seen in the A.M.A. Archives of Surgery. The evidence was accumulated from various sources during a period of more than nine years' clinical and experimental study of the effects of hyper- and hypovolemia.

A DGMS-aided study of 100 consecutive patients undergoing subtotal gastrectomy for benign ulcers at Parkland Hospital, Dallas, affords some of the evidence. For this group, whole blood during their surgery was withheld until there were clear indications of threat from anemia. (Only two of the 100 patients received any whole blood during surgery.) This group was compared with a like series of 99 gastric surgery cases at the Dallas V.A. Hospital who were similarly treated, except that they received blood transfusions ten times as frequently during operation (22 of the 99 received whole blood during operation.)

The reported difference in morbidity and mortality between the two groups was slight. "It would appear from this comparison that failure to replace whole blood losses during operation is not detrimental," Wilson and Adwan write.

They also summarize the results of experimental studies in which survival of animals transfused with compatible whole blood prior to a standardized hemorrhage, was compared with that of controls not transfused prior to the hemorrhage. Only 15 percent of the transfused animals survived, compared with 75 percent of the controls. These and other findings were interpreted as evidence that such transfusion in the absence of an existing physiological need for blood, may add to risk of morbidity and mortality from gastric and other operations.

The Dallas investigators acknowledge that whole blood transfusions are indispensable for treating large blood losses that are inimical to recovery. They suggest maximum discrimination in the selection of such cases, and care in weighing the risks of giving blood against those of not giving it.

SERUM FOLIC ACID ASSAY BY CHEESE BACTERIA VALUABLE FOR DIAGNOSIS OF ANEMIA

Lactobacillus casei, a folic acid-dependent organism common in cheese, is reported capable of using human blood serum as its sole source of this vitamin. The New York City group which reports this finding has developed an assay method based on the growth of this organism in serum samples for use as a diagnostic aid in singling out the cases of megaloblastic anemias which are due to deficiency of folic acid rather than of vitamin B_{12}. Sharp delineation of such cases and their effective treatment with folic acid are reported.

Earlier reports by the New York team that a folic acid-dependent organism (Lactobacillus casei) utilizes the folic acid activity of fasting serum for its growth were received with reservations by some authorities, for there has been doubt that the blood contains any folic acid during the fasting state. An English worker commented in the British Medical Journal that, although some growth of folic acid dependent mutants may occur, "...there is no evidence that this slight growth reflects the presence of folic acid in those blood samples."

The New York (Mt. Sinai Hospital) group advanced their case much further in 1960, reporting that they can relate the growth of L. casei in sera to folic acid nutritional states and that the measure of this growth is useful in distinguishing cases of megaloblastic anemia due primarily to folic acid deficiency from those which are due primarily to vitamin B_{12} deficiency.

Nearly all of the megaloblastic anemias are attributable to deficiencies of either vitamin B_{12} or folic acid or both. Delineation of those due to B_{12} deficiency has been aided by availability of microbiologic assays for this vitamin in serum. The presumed absence of folic acid from the blood has, until now, denied the convenience of such assays to those who wish to assess folic acid nutritional status.

In the hematologic journal Blood, the New York group reports use of this assay for determination of the levels of folic acid active material in normal subjects and in 18 patients with known megaloblastic anemias. The patients included cases of megaloblastic anemia of malnutriton (3 cases), alcoholic cirrhosis (3), infancy (2), pregnancy (2), and of various gastrointestinal diseases (8). Determinations were also done on 10 normal controls, and on patients with various other forms of anemia.

Sixteen of the 18 patients with the known megaloblastic anemias were shown by the assay data to have low serum "folic acid" levels, according to the New York team. In all cases except one, the anemia responded to folic acid administration, and in most the recovery was complete, according to the report in Blood.

Twenty-four sera provided by another lab and representing unknown clinical states were also assayed for "folic acid," and five were found low. The investigators report that in all but one, the "folic acid" level obtained by the assay correlated with the clinical diagnoses which were subsequently obtained.

"While it must be emphasized that the chemical nature of 'folic acid-active' material in fasting serum is unknown," they explain, "nevertheless the quantity of this material appears to reflect the folic acid nutritional status of man."

Participants in the study were Victor Herbert, Herman Baker, Oscar Frank, Inez Pasher, Harry Sobotka and Louis Wasserman of Mt. Sinai Hospital, New York. The work was aided by an NIAMD, as well as a DGMS grant.

DIETARY VARIATIONS AFFECT YIELD OF INBORN DEFECTS FROM VITAMIN E DEFICIENCY

Dorothy Wei Cheng of the State University of Iowa reported, in 1952, the first experimental congenital abnormalities to be produced by vitamin E deficiency. She has since announced a number of findings suggesting interaction of other physiological influences with the vitamin E deficiency in the production of congenital malformations. In 1959 her team reported DGMS-aided

studies showing that the incidence of congenital abnormalities resulting from vitamin E deficiency can be reduced or abolished by treating the deficient mothers with female gonadal hormones (estrone or progesterone). Cheng's experimental animal for these continuing studies is the rat.

In 1960, Cheng's group published a report in The Journal of Nutrition suggesting that dietary constituents, apart from the known vitamin E intake, may influence the occurrence of congenital abnormalities and fetal losses induced by vitamin E deficiency. The new findings were facilitated by the use of synthetic diets, instead of the semi-synthetic ones used previously. (Pure crystalline vitamins A, B, and D were substituted for the natural food materials which had been used as a source of vitamins other than E in the earlier experimental diets.) Contributing dietary factors which were previously beyond effective control thus became amenable to study.

Four diets of different composition (two synthetic and two semi-synthetic) were developed and used in the study. All four were shown to be sufficiently lacking in vitamin E activity to exclude the possibility of fetal survival in any female rats limited to these diets. (In control studies, 100% of the young conceived in females on these diets were found to be reabsorbed.) The diets were then supplemented during pregnancy with just enough vitamin E to maintain the females in a "borderline" vitamin E nutritional state, sufficient to alter normal development of their young without causing their prenatal death.

Twenty different types of congenital abnormalities are listed as resulting from such treatment. These included malformations of viscera, skeletal system, central nervous system, circulatory system, and mouth.

Varying the composition of the diets influenced the yield of abnormal as well as normal young rats, the incidence of fetal resorption, and the average number of implantation sites in the uterus following conception, the investigators report.

Doubling the "borderline" allowance of vitamin E (alpha tocopherol) supplementation during pregnancy resulted in a uniformly significant increase in the percentage of normal young delivered, but even with this doubling of vitamin E allowance, the percentage of abnormal young could be significantly increased or decreased, depending on which of the four rations was used. The substitution of crude casein (milk protein) for the vitamin free casein of a synthetic diet appeared to cause a decrease in the incidence of fetal resorption and an increase in the number of abnormal young and in the number of implantation sites. This is attributed by the authors to the addition of trace vitamins

(presumably other than vitamin E) which are present in the crude casein. The addition of cod liver oil to a pure synthetic ration resulted in a significant increase in the yield of normal young together with a significant decrease in the percentage of resorption.

The hope is that such studies will help expose, for preventive action, interrelated dietary and other factors which may be involved in the causes of human congenital abnormalities.

Thomas Bairnson, A. N. Rao and S. Subbammal participated with Dorothy Wei Cheng in this work.

DECLINE OF SERUM VITAMIN B_{12} IN PREGNANCY RELATED TO WITHDRAWAL BY FETUS

Bacon F. Chow and Kunio Okuda, of Johns Hopkins University, Baltimore, Maryland, have found that the normal fetus draws vitamin B_{12} and other nutrients such as iron and vitamins C and B_6 against a concentration gradient across the placenta with the result that higher serum levels of these substances are maintained on the fetal side of the placenta than on the maternal side. Their report appeared in the Journal of the American Medical Association.

Previous studies had shown a decline in maternal vitamin B_{12} serum levels with advancing pregnancy. The Johns Hopkins investigators demonstrated in tests on 25 women that the umbilical cord serum level of vitamin B_{12} and various other vitamins rises as the result of an active withdrawal from mother to fetus. In view of this fact, it was considered helpful to know whether or not the withdrawal of B_{12} affected the mother. Although no real illness appeared in the mothers, there was biochemical evidence of B_{12} deficiency, based on erythrocyte glutathione and glucose-ribose enzyme content. In another experiment, rats on a standard diet with liver supplements were repeatedly mated. Their maternal reserves became depleted, and it was found that both the size and the number of young were reduced, with each successive pregnancy.

In 14 pregnant women, most of them in active labor, the intramuscular injection of crystalline vitamin B_{12} resulted only in a temporary elevation of the vitamin B_{12} concentration in the maternal blood above that in the fetal blood; after six hours the fetal levels were again higher than the maternal.

To test the nature of the selective transfer mechanism across the placenta, Drs. Chow and Okuda carried out experiments to determine whether all soluble materials could be concentrated in the fetus, in which case the transfer of B_{12} might not be

particularly significant. Tests with glucose and inulin indicated that while they apparently move across the placental membrane via a process of passive diffusion they are not actually drawn across the placenta as in the case of B_{12}. The concentrations of glucose and inulin adjusted to the same levels in the serum of both mother and fetus.

According to the investigators, a factor that points to the physiological importance of the differences in the vitamin B_{12} serum gradient is that in cases of cretinism, the B_{12} concentration in the umbilical cord blood of the cretins is low. This may indicate that the mechanisms of transfer of B_{12} from mother to fetus in cretins is not functioning correctly. Further study is required.

STARVATION MAY PROLONG DRUG ACTION BY SLOWING LIVER ENZYME ACTIVITY

The ability of mammals to metabolize drugs appears to reside largely in the enzymes of the liver cell associated with the endoplasmic reticulum from which the microsomal fraction is derived. Starvation-induced changes, occurring in the endoplasmic reticulum, led a team of pharmacologists at Iowa University to look for corresponding changes in drug metabolism. They now report evidence of marked slowing of drug oxidation along most of a variety of metabolic pathways studied in starved animals.

The Iowa City team used mice for *in vivo* and *in vitro* studies of starvation effects on the side chain oxidation pathway of hexobarbital, N-dealkylation of pyramidon, hydroxylation of the aromatic ring of acetanilid, oxidation of the ring sulfur of chlorpromazine, reduction of aromatic nitro-group of p-nitrobenzoic acid, and reduction of aromatic azo-group of neoprontosil.

The effect of starvation in prolonging hexobarbital-induced sleep was measured in individual animals and then shown to correlate well with biochemical changes in metabolism of most of the drugs by the microsomal fraction of liver cells as determined *in vitro*. In other words, the animals which slept longest under the barbiturate were those whose livers were least able to metabolize most of the drugs studied. The depression of drug metabolism that occurred in most instances is attributed by the investigators to an actual loss of enzyme protein in the microsomal fraction.

In view of these findings, the nutritional status of an animal may be important in accounting for "biological variation" in drug response, according to the investigators. A properly controlled experiment should include regulation of food intake before any drug administration, they suggest. They also believe their findings may have clinical importance where drugs are administered to debilitated or malnourished patients.

Robert L. Dixon, Robert W. Shultice and James R. Fouts of the Iowa group published the data in Proceedings of the Society for Experimental Biology and Medicine.

HIGHLIGHTS OF PROGRESS

IN THE

DIVISION OF RESEARCH GRANTS

1960

Items of Interest on Program Developments

The growth, both in size and scope, of the National Institutes of Health extramural program has again necessitated expansion and reorganization of the many activities of the Division of Research Grants.

In order to coordinate the operation and policies of the complex and growing NIH programs for the support of training of research manpower, the Division was assigned the administration of the NIH training grant and traineeship awards. Responsibilities in this area are: initial receipt of training grant applications with assignment to the appropriate Institute or Division, service as a central source of information in the training grant area and formulation of policies and procedures pertaining to training grants.

To assure maintenance of the Division's high standards of review for the growing number of research grant applications, the number of study sections in the Research Grants Review Branch was increased from 35 to 36, an advisory committee on computors was organized and the position of Deputy Branch Chief was established. The study sections, whose primary function is to provide initial review of research grant application, have also continued their program activities by stimulating and planning an increased number of meetings and institutes to exchange, gather and publish information on specific health and medical problems and needs.

The Health Research Facilities Program began its fifth year of operation in July, 1960. As of October 1, 1960, 212 research facilities constructed under this program were completed and in use. These represent Federal expenditures of $28.6 million and a total construction cost of over $139 million.

The Research Fellowships Review Branch has changed the composition of its review panels so that outside specialists now review and evaluate regular fellowship applications. In the past this process has been carried out by NIH research personnel. Additionally, the number of these disciplinary review panels has been increased from nine to ten to help accommodate the sharp increase in the number of applications. Near the end of 1960 the entire Fellowships Pro-

gram was modified and expanded to meet changing needs of the scientific community. As a result the Branch has been assigned new responsibilities for development of policies, rules and procedures for the Senior Research Fellowship Grants Program and Special Fellowships Grants Program.

During calendar year 1960, the Division reviewed and processed approximately 16,850 research grant applications requesting $338,700,000 and 4,000 applications for research fellowships. This compares with 14,841 research grant applications and 2,848 fellowship applications in calendar year 1959.

FY 1960 RESEARCH AND CONSTRUCTION GRANTS

The National Institutes of Health awarded 11,743 grants totaling $229,505,503, for research and construction of research facilities in nonfederal institutions during Fiscal Year 1960.

Approximately 87 percent of the total amount granted, or $198,719,397, went for support of 11,572 research projects concerned with major diseases and various basic problems in the medical and biological sciences. The grants were made to 973 institutions in the United States and to 145 institutions in 38 other countries.

Grants, totaling $30,786,106, to help build, equip or expand 171 research facilities, were awarded on a matching basis to 141 institutions throughout the country.

DR. ALLEN APPOINTED ASSOCIATE NIH DIRECTOR FOR RESEARCH GRANTS

Dr. Ernest M. Allen, Chief, DRG and Associate Director for Research Grants, NIH, was given full-time staff responsibility as Associate Director. DRG operating responsibilities are now carried out by Dr. Dale R. Lindsay, who was appointed Chief of the Division. His assistant is Dr. Clinton C. Powell, who has been named Deputy Chief.

Dr. Lindsay, formerly Assistant Chief of the Division, has been a career officer of the Public Health Service since 1943. His first assignment in the Public Health Service brought him into malaria control in the War Areas Program. In 1948 he was appointed chief of the Service's field station in Thomasville, Georgia, where he remained until his transfer to the Division of Research Grants in 1953 as Chief of its Program Analysis Section. Two years later he occupied the post of Scientific Director of the Division of Research Grants, and in 1957 the position was renamed as Assistant Chief.

Dr. Powell, formerly Assistant Grants Branch Chief for Clinical Research in the Division of Research Grants, is also a career officer in the Public Health Service. Prior to his Branch Chief position he served as Executive Secretary to the Radiation and Surgery Study Sections. In 1956 Dr. Powell headed the Radiological Health Medical Program, Division of Special Health Services.

FEDERAL AVIATION AGENCY ADVISORY GROUP

Dr. Joseph V. Michalski, Assistant Chief of the Research Grants Review Branch, DRG, accepted membership on the Research Advisory Group of the Bureau of Aviation Medicine, Federal Aviation Agency in September.

This group reviews and evaluates medical research contract proposals submitted to the Bureau.

The group includes representatives of NIH, the U.S. Navy, Army, and Air Force, and Georgetown University School of Medicine.

DRG TRAINING GRANT REVIEW

DRG's Training Section began active review and assignment of all NIH graduate training grant applications to Institute training programs in July.

In February 1960 the Division of Research Grants was designated as the center for administration of NIH training grant and traineeship programs. As a result the Division established during April a new Training Section to be headed by an Assistant Chief for Training, DRG. This position was filled on April 15, with the appointment of Dr. Fay M. Hemphill, who had been serving as chief of DRG's Statistical Design and Analysis Section. Subsequently, Dr. Roy P. Lindgren of DRG was assigned as Project Review Officer, and further staffing brought the Section up to its present complement of eight persons.

The Section's responsibilities in the training grants area parallel DRG's activities in the research grants area: receiving, assigning, and distributing applications; reviewing budget requests of applications; establishing policies and procedures for payment and management of training grants and awards; maintaining central records; establishing training grant review committees where requested; and other duties involving liaison.

FELLOWSHIPS BRANCH REVISES PROGRAMS

The Research Fellowships Review Branch announced the establishment of two new fellowship grant programs, Career Research Professor and Special Fellowship, and the revision of a third, Senior Fellowship.

The Career Research Professor Grant Program was established to support individuals of demonstrated capacity to pursue with distinction a professorial career in independent research and training. Grants are awarded initially for five years of support and are renewable at five-year intervals. In this first year the award will be made only to schools of medicine, osteopathy, dentistry, and public health. The earliest effective beginning date is July 1, 1961.

The Special Fellowship Grant Program provides support for promising young investigators, not yet eligible for a higher level, who the applicant institution feels would be an important addition to its teaching and research staff. This grant program is in addition to the present Special Fellowship awarded directly to individuals for advanced or special training.

The Senior Fellowship Grant Program, formerly limited to preclinical science departments, has been expanded to include clinical departments and certain departments in university graduate schools and other institutions. This program is for the support of individuals with at least five years of relevant research experience beyond the doctorate, who have demonstrated high potential for a research or academic career.

ADVISORY COMMITTEE FOR COMPUTERS IN RESEARCH

An Advisory Committee for Computers in Research, composed of biologists, physicists and experts in computer technology was established in DRG in September.

The Committee has four general functions: (1) to advise the NIH on the role of computers in medical and biomedical research, areas of medical research in which computer usage might be encouraged through NIH efforts, and areas of research in computer technology which should be stimulated and supported by NIH activities; (2) to appraise and assign priority scores to applications for grants referred to the committee for such actions; (3) to appraise and prepare recommendations on applications and inquiries referred by Study Sections; (4) to stimulate use of computer techniques in medical research and development of computer technology as applied to biomedical problems.

Chairman of this new nine man committee is Dr. Lee B. Lusted of the University of Rochester. Other members are: Mr. Paul Armer, The Rand Corporation; Dr. Mary A. B. Brazier, Massachusetts General Hospital; Dr. David Garfinkel, New York State Psychiatric Institute; Dr. George A. Sacher, Argonne National Laboratory; Dr. Otto Schmitt, University of Minnesota, Department of Physics; Dr. Norman Shapiro, National Institutes of Health, Division of Research Services; Dr. Ralph W. Stacy, Ohio State University, Department of Biophysics; Dr. Max Woodbury, New York University, School of Engineering.

The first general meeting of the Committee was held at NIH September 20 and 21.

THIRTY-SIXTH STUDY SECTION ESTABLISHED

On May 27, the Division of Research Grants received approval of the Director, NIH, for the establishment of the Division's 36th Study Section -- Medicinal Chemistry.

The new Study Section was formerly DRG's Panel of Medicinal Chemistry Consultants. Dr. Helen Jeffrey, who served as Executive Secretary to the Biochemistry Study Section and to the former Panel, continues in that capacity for both the Biochemistry and Medicinal Chemistry Study Sections.

CONFERENCE ON "PHENOMENA OF TUMOR VIRUSES"

A symposium entitled, "Phenomena of the Tumor Viruses," sponsored by the Virology and Rickettsiology Study Section of DRG, was held in New York City in March.

Recent findings in experimentation with the tumor viruses were presented in papers read by twelve U.S. and foreign scientists, who have done outstanding work with viruses in various disciplines. Each presentation served as a basis for questions and informal discussion. General categories are "Properties of Tumor Viruses," "Host Response," "Contributions of Tissue Culture," and "Ultrastructure."

Speeches by two NIH administrators served as an introduction to the two-day meeting. Dr. Joseph E. Smadel, Associate Director for Intramural Research, discussed the "Tumor Virus Research Program," and Dr. Ralph G. Meader, NCI Research Grants Branch Chief, outlined "Administration of Research Support."

About 200 investigators actively engaged in work with tumor viruses participated in the meeting, supported by a research grant from the National Cancer Institute.

Dr. J. W. Beard, Duke University, was chairman of the symposium.

CONFERENCE ON "PROBLEMS IN TOXICOLOGY"

DRG's Toxicology Study Section, in cooperation with the Food Protection Committee and the Toxicology Committee of the National Research Council, co-sponsored a symposium on "Problems in Toxicology" in Chicago.

The symposium was an intersociety meeting held in conjunction with the Annual Meeting of the Federation of American Societies for Experimental Biology.

The purposes of the symposium were to attract graduate students to toxicology, to encourage competent investigators to undertake research in this field, and to discuss the ever-increasing number of problems in this area.

Leading scientists talked about relatively new challenges in the field, such as "Radioactive Materials," "Submarine Hazards," "Space Hazards," and "Man-made Noxious Environment." Other topics were "Food: Additives and Natural Components," "Drugs and Cosmetics," "Agricultural Chemicals," "Water and Atmosphere," "Recognition of Toxicology as a Scientific Discipline," and "Training of Toxicologists."

Chairman of the morning session of the day-long symposium was Dr. Chauncey D. Leake, Professor of Pharmacology, Ohio State University. Dr. W. Henry Sebrell, Jr., Director of the Institute of Nutritional Sciences, Columbia University School of Public Health and Administrative Medicine, chaired the afternoon meeting.

ADVISORY COMMITTEE FOR SCIENCE EXPOSITION

Dr. Ernest M. Allen, DRG Chief, is serving on a committee of advisors to the Commissioner for the U. S. Science Exhibit, Century 21 Exposition, to be held in Seattle in 1961.

The group, called the U.S. Science Exhibit Advisory Committee on Scientific Theme, Content and Presentation, is composed of nine experts in the field of science and mass communications. This body makes recommendations on specific exhibits and methods of presentation, scientific integrity of the exposition, and overall theme.

Also serving on the Committee are representatives of the Department of Defense, National Bureau of Standards, American Association for the Advancement of Science, Department of Agriculture, Harvard University Observatory, National Science Foundation, University of Pennsylvania Museum, and National Aeronautics and Space Administration.

TWO SYMPOSIA ON NUTRITION

The DRG Nutrition Study Section cosponsored two symposia, "Problems of Nutrition Appraisal of Mankind," and "Interaction of Mineral Elements in Nutrition and Metabolism," in Chicago. The sessions were held in conjunction with the Annual Meeting of the Federation of American Societies for Experimental Biology.

The morning symposium, "Problems of Nutrition Appraisal," was cosponsored by the Interdepartmental Committee on Nutrition for National Defense. Investigators representing the fields of medicine, anthropology and biochemistry conferred on "Problems of Physical Appraisal," "Dietary Appraisal," and "Problems of Biochemical Appraisal of Nutritional Status." Dr. John B. Youmans of the Surgeon General's Office, U.S. Army, served as Chairman.

Papers given in the afternoon symposium, "Mineral Element Interaction," illustrated the close interrelationship of mineral elements in the living animal body. The American Institute of Nutrition co-sponsored this meeting, chaired by George K. Davis, Professor in the Department of Animal Husbandry and Nutrition of the University of Florida. Topics explored were "Calcium and Phosphorus Interactions in Nutrition and Physiology," "Nutritional Interactions in Zinc and Calcium," "Magnesium Requirement and Its Relation to Other Dietary Constituents," "Metabolic Relations of Manganese to Other Minerals," "Interrelationships of Iron and Copper in the Nutrition and Metabolism of Animals," and "Interrelations of Copper, Molybdenum, and Sulfate Sulfur in Nutrition.

FOURTH CONFERENCE ON RESEARCH NEEDS IN TROPICAL MEDICINE

The Fourth Conference on Research Needs in Tropical Medicine was held at the School of Medicine, Louisiana State University in New Orleans. Similar conferences in this field were sponsored by the predecessors of the Tropical Medicine and Parasitology Study Section in 1951, 1953, and 1954.

Chairmen of the Fourth Conference was Dr. Stanley B. Freeborn, Chairman of the Tropical Medicine and Parasitology Study Section, and Dr. William W. Frye, Vice President of Louisiana State University and Dean of the School of Medicine.

The purpose of the informal, invitational conference was to explore the status of research in tropical medicine, with emphasis on the interdisciplinary areas, to better understand the current needs in the wide scope of tropical or geographical medicine; to broaden the concept of tropical medicine to geographical medicine (which includes all disciplines in addition to infectious and parasitic diseases); and to better orient subsequent research in geographical medicine.

Many of the participants were PHS grantees or potential grantees. Participants represented universities, leading pharmaceutical laboratories, laboratories of private foundations, private research institutions, World Health Organization, International Cooperation Administration, U.S. Department of Agriculture, the U. S. Army, Navy, and Air Force, Veterans Administration, and the Public Health Service.

The program was composed of three panels: "Tropical or Geographical Medicine," "Impact of Modern Instrumentation on Medicine in the Tropics," and "Critical Problems Needing Investigation in Some Fields of Medicine in the Tropics." It was designed to encourage questions

and informal discussion following the presentation of each topic. Some of the topics considered were: tropical pathology; clinical periodontal, neurological, nutritional, cardiovascular, infectious and parasitic diseases; population limitations; cancer; mental health; microbiological techniques and electron microscopy; zoonoses; immunology and fluorescent antibody techniques; biochemistry; and histochemistry. Some 15 other subjects were suggested but not included due to the lack of time; thus, a fifth conference has been recommended for 1961.

The fourth conference was supported in part by a research grant awarded to the Louisiana State University (Dr. William W. Frye, Principal Investigator) by the National Institute of Allergy and Infectious Diseases. Dr. Ernestine B. Thurman serves the Tropical Medicine and Parasitology Study Section as Executive Secretary.

Proceedings of the conference were published in September.

GENETICS STUDY SECTION SPONSORS THREE SYMPOSIA

The DRG Genetics Study Section sponsored the first in a series of three symposia on "Methodology in Genetics" at the University of Utah, Salt Lake City.

A recent survey by the study section indicated a critical lack of information and communication concerning current methods in rapidly advancing areas of medical genetics. These symposia bring such information up to date and will make it readily available to the genetics research community through low-cost, widely distributed publications. The meetings are supported by a grant from DGMS.

The first symposium, concerning "Human Genetics," featured papers by fifteen outstanding geneticists. The subject groupings of these papers were "Analysis of Human Heredity," "Genetics and Disease," "Mutations in Human Population," "Cytogenetics," and "Biochemical Genetics." Each group of papers was followed by an informal discussion. The second symposium, "Methodology in Mammalian Genetics," was held at the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

Thirteen papers were presented on various problems such as "Genetics Stocks and Breeding Methods," "Radiation Genetics," "Physiologic Genetics," "Biochemical Genetics," "Immunogenetics," "Host Parasite Relationships," and "Somatic Cell Genetics."

The third meeting, "Methodology in Basic Genetics," will be held at the University of Texas, Austin, on May 4-5, 1961. The study section plans to distribute the publication of proceedings from the first symposium at this third meeting.

SUMMER INSTITUTE IN BIOPHYSICAL SCIENCE

The Biophysics and Biophysical Chemistry Study Section, DRG, sponsored a "Summer Institute in Biophysical Science" at Massachusetts Institute of Technology.

College undergraduates, majoring in chemistry, physics, mathematics, biology or in premedical studies, attended the Institute as representatives of sixteen universities and colleges throughout the nation.

The purpose of the two-week program was to stimulate and encourage participation in the development of the broad field of biophysics. Lectures on biophysics, a talk on "Education and Career Opportunities in Biophysics," laboratory tours and a trip to the Marine Biological Laboratory at Woods Hole, were included in the two-week program.

CONFERENCE ON PROSTHETIC VALVES FOR CARDIAC SURGERY

DRG's Surgery Study Section sponsored a "Conference on Prosthetic Valves for Cardiac Surgery," at the Edgewater Beach Hotel, Chicago, Illinois. The Conference was supported by a grant from NHI.

Introductory remarks to the two-day session were made by Dr. James Watt, Director of NHI. Almost thirty professional papers were presented by U. S. and Canadian heart specialists, including several from NHI. A discussion, led by an expert in the field, followed each group of papers, arranged in such topics as "Physiology of Valve Function," "Myocardial Metabolism," "Materials, Designs and Testing Methods," "Replacement of the Mitral Valve," and "Replacement of the Aortic Valve."

CONFERENCE ON THE PHYSIOLOGICAL ASPECTS OF WATER QUALITY

DRG's Environmental Sciences and Engineering Study Section sponsored a "Conference on the Physiological Aspects of Water Quality" in Washington, D. C.

The two-day program was divided into a general session, "Session on Minerals and Trace Elements," "Session on Insecticides and Organics," and a review session. The general session included discussions on "The Need for Water Quality Criteria" and "Detection and Analysis of Chemistry in Water."

The review session included summaries on minerals and trace elements, insecticides, organics, "National and International Standards of Water Analysis," and a conference evaluation.

About 65 scientists, representing Federal agencies, State Health Departments, Universities and private organizations throughout the U.S., participated in the Conference.

REVISION IN PROCEDURE FOR GRANT PAYMENT

NIH 1961 grants, with certain exceptions, will be paid in four installments.

The NIH grant payment procedure was changed, as of July 1, to minimize excessive cash balances in the accounts of grantee institutions in proportion to their current operating needs.

ALMA MATERS OF PRINCIPAL INVESTIGATORS

A tabulation of the Alma Maters of 1900 NIH-supported principal investigators shows that the grantees obtained their bachelor's degrees from 362 universities representing every State in the Union except Nevada.

The study, made by DRG's Statistics and Analysis Branch, was limited to investigators who received research grants in FY 1960, and who attended institutions located in the United States and Puerto Rico.

The institution graduating most grantee principal investigators was the University of California, followed by Harvard, Chicago, City College of New York, Minnesota, New York University, Columbia, Yale, Michigan, Illinois, and Cornell.

PRINCIPAL INVESTIGATORS ON TEACHING STAFFS

A study conducted by DRG's Statistics and Analysis Branch reveals that of the principal investigators receiving their first research grant in Fiscal Year 1960, 80 percent were on the staffs of schools and universities.

Ten percent were on hospital staffs and the remaining investigators were affiliated with other types of institutions.

The tabulation also shows that of the 80 percent in schools and universities, three-quarters hold rank as assistant, associate, or full professors.

BIRTHPLACE OF GRANTEE INVESTIGATORS

A recent study on the birthplace of some 1400 principal investigators receiving their first research grant in Fiscal Year 1960 reveals that 21 percent were born in foreign countries.

The study, conducted by DRG's Statistics and Analysis Branch, also shows that 12 percent of these 1400 investigators are not U.S.A. citizens.

Other study findings indicate that 40 percent of the native-born new principal investigators were born in New York, Illinois, Pennsylvania, California or Massachusetts. Two and one-half times as many were born in New York as in Illinois or Pennsylvania -- States rated second in the above list.

FINANCIAL AID TO STUDENTS INCREASES

Although college tuition costs have risen sharply during the past few years, there has also been an increase in organizations providing financial aid to students.

The Statistics and Analysis Branch, DRG, found that 86% of NIH's FY 1960 principal investigators aged 30 or younger obtained some formal support (U.S. Government assistance, university scholarship, etc.) during their predoctoral work. Only 40% of those 60 or older had had formal support. For other age groups, the proportions obtaining formal educational support were: Aged 30-34, 81%; aged 35-39, 69%; aged 40-44, 67%; aged 45-49, 62%; aged 50-54, 54%; aged 55-59, 53%.

NIH RESEARCHERS WIDELY EXPERIENCED

More than 25% of some 1,400 principal investigators receiving their first NIH research grant award in FY 1960 have served in the past as assistants on research projects supported by NIH. Two-fifths of the group have been principal investigators for research projects sponsored by other agencies or institutions.

The Statistics and Analysis Branch, DRG, reports that the new investigators receiving support from the National Institute of Arthritis and Metabolic Diseases, as a group, had the widest experience in formal research projects. Almost 3/4 participated previously in sponsored research. In the comparatively new field of dental research almost 50 percent of the new investigators have participated in sponsored research as principal investigators or assistants before obtaining NIH support.

DR. ALLEN ADDRESSES ROCKET SOCIETY CONFERENCE

Dr. Ernest M. Allen, Chief of the Division of Research Grants and Associate Director of NIH, spoke at the American Rocket Society's "Conference on Anatomy of Manned-Space Operations," in Dayton, Ohio.

The three-day Conference was sponsored by the ARS Human Factors and Bio-Astronautics Committee in cooperation with the Aerospace Medical Laboratory and Office of Surgeon, Air Materiel Command, Wright-Patterson Air Force Base and the ARS Dayton Section.

Dr. Allen's talk was part of a panel discussion on "Government Contracts, Grant and Research Interest in the Biosciences Area." Also participating on this panel were representatives of the Army, Air Force, Navy, Department of Defense, NASA, and A.E.C.

Dr. Allen discussed those programs of the NIH that can provide support of research activities in space medicine and other related fields. He cited examples of currently NIH-supported projects in these fields which were of special interest to this audience.

Other sessions of the Conference dealt with "Anthropometrics," "Bioinstrumentation," "Bionics," and "Human Factors in the Conquest of Space."

DHEW REPRESENTED ON FEDERAL RADIATION COUNCIL

Dr. Clinton C. Powell, Assistant DRG Grants Branch Chief for Clinical Research, was appointed by PHS Surgeon General Leroy Burney to serve on the Working Committee of the interagency Federal Radiation Council.

Dr. Powell, a Public Health Service expert in the field of radiation, is the representative of the Department of Health, Education, and Welfare on the Working Committee of the Council. Established by Executive Order last August, the Council began work on January 4 to formulate policies and make plans for investigation into the field of radiation damage, exposure, and health hazards.

Before taking his present post in March 1959, Dr. Powell was Executive Secretary of the Radiation and Surgery Study Sections. He came to DRG in 1958 from the Division of Special Health Services, where he was Chief of the Radiological Health Medical Program.

Dr. Powell is a commissioned officer in the Public Health Service. Posts he has held at NIH include: Grants Analyst, Research Grants and Fellowship Branch, NCI; Staff Physician, Radiation Therapy Branch, Clinical Center; and Radiation Safety Officer.

DR. LINDSAY SPEAKS AT FLORIDA CONFERENCE

Dr. Dale Lindsay, Assistant DRG Chief, participated in a "Conference on Disease in Early Life," in Miami Beach, Florida.

Dr. Lindsay was the Government's spokesman in a symposium, "Crossroads of Research," a discussion of the integration of support of medical research by Government, industry and private endowment. Dr. Lindsay outlined Federal support of biological and medical sciences by research grants, training of scientific manpower, and assistance in the construction of health research facilities.

He also cited examples of Government-industry cooperation and of liaison between the Government and voluntary organizations.

Other panel members were Truman G. Blocker, University of Texas Medical Branch, Galveston, Texas; H. F. Zoller, Ethicon Company, Somerville, New Jersey; and J. M. Weir, Rockefeller Foundation, New York. Chairman of this session was the Honorable Claude Pepper, former U. S. Senator.

This Conference was sponsored by the Variety Children's Research Foundation in honor of the January 10 dedication of the Variety Children's Research Foundation Laboratories in Miami. An NIH grant, awarded on a matching fund basis, made possible the construction of this building. NIH officials attending the dedication were Dr. Justin Andrews, Director of National Institute of Allergy and Infectious Diseases; Dr. Robert J. Huebner, Chief of Laboratory of Infectious Diseases; Dr. W. Ray Bryan, Head of Virus Oncology Section, National Cancer Institute; and Dr. Lindsay. Dr. Huebner and Dr. Bryan also took part in the two-day Conference.

HIGHLIGHTS OF PROGRESS

IN SUPPORT OF RESEARCH

AT NIH

1960

Items of Interest Concerning Major Research Studies and New

Program Developments in the Division of Research Services

A variety of technical, scientific, and engineering services are supplied to scientific personnel at NIH and the Institutes as a whole through the Division of Research Services. The 850 employees who perform these services represent a high degree of skill and creative talent plus a wide range of professional and subprofessional specialties.

The Division operates an animal hospital, animal receiving center, and animal farm; it breeds conventional laboratory animals and produces inbred and exotic strains for use in special research projects. The Division has pioneered efforts at NIH to produce germfree animals on a large scale and offers technologic, advisory, and maintenance services to NIH investigators who are using these animals in their studies.

The Division also serves NIH investigators by supplying the bacteriological and tissue culture media they use, by washing and sterilizing test tubes, pipettes and other laboratory glassware, and by producing specialized glassware that is not available commercially.

Electronic data processing for the scientific and administrative programs at NIH is available centrally in the Division. Instrument engineering and the design and modification of equipment for special-purpose application in medical research are also part of its many-sided service program. The design of exhibits for display at national and international scientific meetings, all phases of medical illustrating and photographic services, scientific translation and interpreting, and the administration of a unique medical library are other research services. A major program is continuous planning by architects and engineers for the location and construction of new research buildings and the remodeling of existing laboratories for new uses.

Frequently, individuals or teams of scientists and technicians in the Division collaborate with Institute investigators on research projects under way, particularly in the service aspects of that research. The Division maintains the high quality of its many services by anticipating the need for and initiating studies relating specifically to the services requested. The cooperative or individual research thus undertaken has a direct application to the research programs of NIH and often results in publication in technical and professional journals or in the form of guides, texts, and manuals. The findings of these studies are thus shared with the scientific public, with other institutions, and with industry.

FRESH EGG YOLKS YIELD RELIABLE TEST FOR ISOLATING "STAPH" ORGANISMS

Among new DRS achievements in environmental sanitation is a simple egg yolk test that has proved to be a sensitive and dependable method for detecting the ubiquitous, and highly adaptable staphylococcus organism. Dr. Lloyd G. Herman, of the Sanitary Engineering Branch, reported successful use of the test to the Society of American Bacteriologists last spring.

A modified commercial medium, in which fresh egg yolks are the essential added ingredient, is used for the primary isolation of Staphylococcus aureus organisms found in clinical specimens and in surgical and patient care areas. The presence of virulent "staph" is readily observed in the strong pigmentation of the colonies, ranging from light yellow to vivid orange, with marked "halos" in the medium.

Because egg yolks are usually sterile, the production of the egg yolk medium is less troublesome than many other enriched media. The modified medium also permits the direct identification of coagulase-positive staphylococci found on routine swabs taken from floors, instruments, sinks, and other sources that tend to be heavily contaminated with other types of bacteria. The high high salt content of this medium tends to slow down the rapid growth of the gram-negative species but permits normal growth of the staphylococci.

MILITARY TECHNIQUES APPLIED TO STUDY OF BIOLOGICAL SYSTEMS

At a recent symposium, Dr. Norman Z. Shapiro, Chief, Computation and Data Processing Branch, and Richard J. Podolsky, Naval Medical Research Institute, reported on a collaborative effort in connection with computer models simulating biological activity. Two models of muscular contraction are under investigation, the "sliding" and "folding" hypotheses. Digital computer programs have been written which implement both of these models. Compu-

tations employing these programs are then used to verify the agreement of each of the two models with experimental observations, thus obtaining indications of the veracity of the models.

The techniques employed are believed to be applicable to a large variety of models of biological systems. They stem from variations of techniques used to implement models of military activities in which a large number of defensive and offensive military elements interact with one another.

NIH ACQUIRES RURAL ACREAGE FOR DEVELOPMENT AS ANIMAL FARM

Late in 1959, NIH negotiated an option to purchase 513 acres of farmland in western Maryland about 25 miles from Bethesda. Title to the large tract, known locally as the "Cider and Ginger Farm," was acquired in April 1960. The acreage replaces a small temporary farm where large animals, such as goats, sheep, horses, and cows, have been held for research studies by Institute investigators.

Planning for future occupancy of the farm and its development as a unified research facility began immediately after purchase. A master site plan developed during the year was approved by the Research Facilities Planning Committee. The plan was prepared by the Research Facilities Planning Branch with the assistance of a special consultant employed by the Division of Research Services. Essentially, the plan is a set of guiding principles based on Institute needs and preliminary surveys of engineering problems that could affect the siting of buildings.

ANIMAL WASTE DISPOSAL STUDIES AID DESIGN OF FARM FACILITIES

As an aid to designing sewage treatment facilities for the NIH Animal Farm, the Sanitary Engineering Branch has completed animal waste disposal surveys at NIH to measure the quality and quantity of waste water discharged during the washing and rinsing of animal cages and kennels, the concentration of detergent in parts per million, and the composition of wet and dry waste production from large and small animals. In related studies at the farm, baseline information has been obtained evaluating the extent of present pollution in the stream passing through the farm to calculate its future capacity for receiving treated liquid wastes.

VARIABLE PROJECTION APPARATUS MAGNIFIES 95X ON FLAT SURFACE

Two models of a unique device for "mapping" areas of the human brain have been developed for the National Institute of Neurological Diseases and Blindness by the Instrument Engineering and Development Branch.

The device is a variable projection system for human neuroanatomical reconstruction in research on brain degeneration. It magnifies up to 95 times and projects slide mounted microscopic sections of cortical tissue onto a flat horizontal surface, such as a desk or table top. Heat damage does not occur to the tissue specimens. Distortion of the projected image is avoided by means of double-reflecting mirrors, optical lenses of varying focal lengths, and a modification of a commercially designed projector.

The design, construction, use, and magnification (5X to 40X) of the prototype apparatus have been described in The Anatomical Record. The substitution of more powerful lenses and the addition of an effective cooling system extended the magnification of the second model to the range of 4X to 94X.

No major modification is required to use the system in other research areas or for medical illustrations, training demonstrations, and allied educational purposes. The projected image provides sufficient illumination so that a completely darkened room is unnecessary.

INGENIOUS DRINKING FOUNTAIN INSTALLED IN ANIMAL HOSPITAL

An innovation in the animal world is the automatic drinking fountain designed for cats and dogs in the Animal Hospital by Dr. William I. Gay and John Carter, and reported in the Journal of the American Veterinary Medical Association. The fountain is a stainless steel basin swinging from a metal bracket attached to a water pipe in an indoor run. A constant supply of fresh water is available through a modified commercial valve assembly. As the water level in the basin drops, a spring opens the valve, and the basin fills with water. A metal cap above the bracket protects the valve from licking or soiling. The fountain is standard equipment in the Animal Hospital, where it has proved to be economical and efficient.

PHOTOCOPIER IN LIBRARY INCREASES USEFULNESS TO SCIENTIFIC PUBLIC

With the set of a dial and the push of a button, a scientist may acquire his personal copy of the journal article he has just read before the journal is released for shelf circulation. An automatic photocopier has been installed in the NIH Library to print photo-exact copies of textual materials on a self-service basis. Originals may be of any color and any thickness, a feature permitting the copying of any page of a bound book. The new service holds promise for increasing the usefulness of the Library's collections of biomedical texts and journals.

NEW RESEARCH FACILITIES AT NIH HAVE UNUSUAL DESIGN FEATURES

Nineteen sixty was a peak year in the accelerated building program begun in 1958 to expand and consolidate research facilities at NIH.

Laboratories completed for the Division of Biologics Standards and nearing completion for the National Institute of Dental Research represent an advance in research facility design.

The DBS Building incorporates in its design special animal facilities, including a monkey-testing area on the top floor. The NIDR Building has a laboratory for germfree animal research. High-pressure air-conditioning systems in each building have filtering devices to trap airborne bacteria. Both buildings have permanent cold and constant temperature rooms, sterile rooms equipped with incinerated air exhaust systems, and movable metal partitions between offices and laboratories so that space can be increased, diminished, or exchanged as needed. Movable partitions have been a custom feature in NIH buildings since the construction of the Clinical Center.

Also approaching completion was the surgical wing to the Clinical Center. The new addition will serve as a guide for the design of surgical research facilities throughout the world. A circular structure, with circular operating rooms and wedge-shaped auxiliary rooms, the wing is devoted to cardiac surgery and neurosurgery.

The cardiac surgeries have glassed-in observation domes equipped with television and motion picture cameras. All of the surgeries will be equipped with surgical consoles. These are mobile, stainless steel units connecting the operating tables with central utilities and piped-in supplies of compressed air, oxygen, nitrous oxide, carbon dioxide, helium, and nitrogen.

The air-conditioning system forces air downward from above the operating table to a floor vent underneath. The flooring is a special, non-conductive latex. Hypothermia and blood bank facilities are included in the structure.

Allied construction activity includes a general office building and the new home for the National Library of Medicine, to be located on NIH grounds.

PORTABLE AIR SAMPLER USED IN NIH ENVIRONMENTAL STUDIES

The Instrumentation, Engineering and Development Branch and members of the Sanitary Engineering Branch have modified a portable air sampler originally developed at Ft. Detrick, Md, for use in air pollution studies conducted by the Sanitary Engineering Branch. The sampler is a versatile apparatus for determining the bacterial content of

air anywhere on the NIH reservation. It allows continuous air sampling for either two or eight hour runs at a controlled rate of 15-30 liters a minute. The bacteria are deposited onto a 20-inch long agar-filled tray driven by a motor past a stationary slit. After the tray run is completed, the tray is removed for incubation and later observation of bacterial growth.

The portable sampler is highly adaptable and may use the existing vacuum service in a building or its own mechanical pump. It may be connected to an existing standard 115-volt alternating current line, or, if such electrical service is not available, it can operate from its own 12-volt storage battery. This battery is capable of running both the vacuum pump and the tray driver motor for an 8-hour period.

GENETIC TESTING IS ROUTINE TECHNIQUE IN INBRED MOUSE BREEDING COLONY

Last year the Laboratory Aids Branch announced a rapid skin-grafting technique to determine the genetic purity of inbred mice, and in October it began using the technique for routine testing of mice in the expansion breeding colony for inbred strains. The expansion colony is intermediate between foundation and production colonies of inbred strains. Any difference in genes resulting from errors in mating or from mutations within the strain can be quickly detected with the skin-grafting technique. Tail skin from a female in each new litter is grafted onto the tail of an ex-breeder of the same pedigreed strain. If the graft takes, it can be assured that all siblings will possess the desired genetic characteristics as the breeding stock and that genetic contamination has not taken place. Potentially, skin-grafting can be used as a routine check to determine the genetic homogeneity of all inbred mice issued to NIH investigators.

This technique is described by Dr. Donald W. Bailey and Bashiruddin Usama in "A Rapid Method of Grafting Skin on Tails of Mice," Transplantation Bulletin.

DICTATING EQUIPMENT RECORDS ORAL SCIENTIFIC TRANSLATIONS

The use of modern dictating and transcribing equipment has been extended to the recording of scientific translations. Previously it was customary for a translation of a scientific paper to be drafted, edited, and reproduced in multiple copies by the translating service of the NIH Library. Now, a translator can bypass the production of written translations by orally translating sufficient information for a scientist to assess research progress in a foreign country that may relate to NIH investigations. The recorded translation becomes his permanent property, to use at his convenience.

AIR-CONDITIONED ANIMAL TRANSPORT REDUCES HEALTH HAZARDS

An animal van used to transport the dogs and cats donated by humane societies to the Animal Hospital was air conditioned last summer to prevent extremely high temperatures within the vehicle. Air-conditioned transport has kept the animals free of the usual discomforts and health hazards associated with excessive heat and close quarters.

COST DISADVANTAGE OF DISPOSABLE TEST TUBES OFFSET BY CONTAMINATION FACTOR

In response to numerous requests, the Laboratory Aids Branch issues one-use clear glass tubes to investigators who prefer a disposable tube for certain flocculation and complement fixation tests. The cost of the new tube is about half the standard multi-use tube, but the high cost of use is offset by the absolute certainty that no organic matter is in the tube, and the assurance that any previous use of the tube can in no way affect current experimental results. Another advantage is the saving of the time and trouble in having to pick over a mass of multi-use standard tubes to select only those clear enough for the tests mentioned.

LOW-COST PLASTIC SPLINTS MAY REPLACE PLASTER OF PARIS

From time to time, the Medical Arts Section collaborates in certain phases of Institute research programs, and as with all research efforts, trial-and-error attempts are frequently necessary before an entirely satisfactory solution is found.

As an example, the Section is working with the Rehabilitation Department of the Clinical Center in its search for corrective or supportive splints that promise a longer life than that afforded by plaster of paris. More than 200 plaster casts or splints are applied to patients annually at NIH. Even though plaster has proved to be extremely effective, there is need for durable splints that cosmetically are more acceptable than plaster. The ideal splint would also be low in cost, light in weight, and easily cleanable. Toward this end, the Department is working with a foam plastic that holds great promise.

In the Medical Arts Section, steps have been taken to develop a plastic wrist splint, which, while it may not prove to be the answer, has provided extremely useful developmental experience. A prototype splint has been made of a plastic blend of styrene and rubber. As a prosthetic device, a styrene-rubber splint possesses several obvious advantages over plaster and the heavier plastics. The splint may be easily removed. Flocking on the interior surface makes the splint comfortable to wear. The plastic is both lightweight and opaque and while it is not flexible, neither is it rigid. Its cost is low, and its fabrication as a splint is so

simple that anyone could produce it after seeing a demonstration.

New findings from this search for improved splints and casts will aid the rehabilitation of persons suffering from rheumatoid arthritis. Dr. David Fried, Chief of the Rehabilitation Department, initiated the program.

SPECIAL ENVIRONMENTAL CHAMBERS DESIGNED AND CONSTRUCTED

A wide variety of unique environmental chambers required for the NIH research programs have been in the design and construction stage during the past year. Research requirements for these facilities have challenged the technical skills and ingenuity of the Plant Engineering Branch, which provided the designs and supervised or carried out the constuction program.

Plant Growth Chambers. Growth chambers constructed for the National Institute of Mental Health were designed to determine the optimum conditions under which plant life achieves a high enzyme and akla-loid yield. Temperature, humidity, air movement, and air contamination are controlled precisely, with a minimum of variation permitted. The illumination is of a very high intensity, nearly 4,000 foot-candles, to simulate the shady side of a house on a bright day. The information sought may have an important bearing on the role of enzymes and alkaloids in the development of nerve tissue.

Low-Level Radiation Counting Facilities. A low-level radiation counting facility, where "counting" devices and other instruments can record the amount of radioactivity within the human body and other objects without influence from the immediate surroundings, was constructed for the National Cancer Institute. The facility is a physically isolated chamber in which background radiation--from the air, construction materials, instruments, and other room objects--is reduced to a minimum. Isolation of the chamber from the rest of the building is achieved by complete recirculation of the air; airborne contamination from recirculating air is further reduced by filtering the air. The walls, floors, and ceilings of the "counting" cubicles are constructed of steel six inches thick.

Hydrogenation Laboratory. A hydrogenation laboratory for the joint use of all Institutes was built with safety in mind. Its primary purpose is to provide safe operation for the high-pressure hydrogenation processes used in various research programs. One wall of the building, which is an extension to the Isotope Laboratory, faces a natural earthen barrier and is constructed of $\frac{1}{4}$-inch cement-asbestos board that will give way in the event of high pressures building up within the building itself. As further protection, all electronic equipment is subject to remote control.

Sound-Proof Test Rooms. The construction of independent, enclosed rooms in which NIMH can perform basic research tests on animals, particularly monkeys, without interference from external noise or vibration, utilized plywood, sand, isolation pads, and other standard materials to obtain signification reduction (55 decibels) in sound transmission through walls, floors, and ceilings.

Blood Serum Cold Storage Rooms. Serum storage rooms to provide highly dependable and maintenance-free facilities for storing 600,000 vials of human blood serum at a constant temperature of -20°C were designed for the collaborative cerebral palsy studies of the National Institute of Neurological Diseases and Blindness. To assure uninterrupted frost-free operation for a minimum of 10 years, duplicate refrigeration machines, hot-gas defrost systems, and emergency sources of electrical service and cooling water were installed.

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